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Med Pregl 2022; LXXV (Suppl 1): 1-172. Novi Sad

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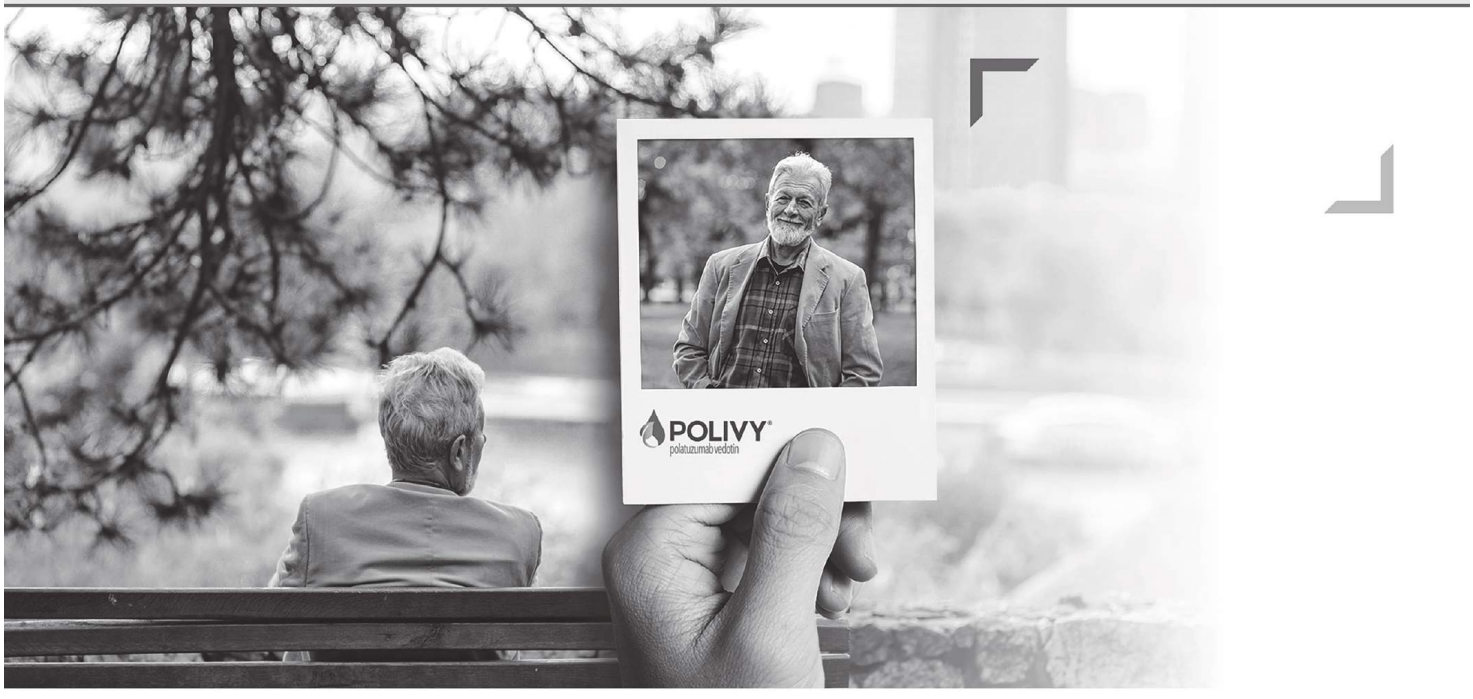

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Dear colleagues,

It is our great pleasure to invite you to participate in the 5th Congress of Serbian Hematologists with international participation, which will be held from October 6th to 9th, 2022 in Novi Sad. It is not a coincidence that Novi Sad was elected to be the place of the traditional meeting of Serbian hematologists in 2022, since it is the year our city is The European Capital of Culture. With the high-quality program, we would like to merge the experiences, as well as exchange and enrich our knowledge in hematology with colleagues not only from Serbia but also from neighbouring countries as well as other European countries.

Topics covered by the Congress will be contemporary, interesting and inspiring. We will try our best to make the meeting more interactive through case reports, debates, and open discussions. Particular attention is going to be devoted to young hematologists as they are future developers of hematological knowledge.

We wish you a valuable and exciting participation in the congress and a pleasant stay in Novi Sad.

On behalf of the organizing and scientific committee

Prof. dr Ivana Urošević

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CURRENT STRATEGIES FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA

SAVREMENI VIDOVI LEČENJA AKUTNE MIJELOIDNE LEUKEMIJE

Nada SUVAJDŽIĆ VUKOVIĆ[1,2], Mirjana MITROVIĆ[1,2], Marijana VIRIJEVIĆ[1,2], Ana VIDOVIĆ[1,2] and Zorica CVETKOVIĆ[3]

Summary

Introduction. Acute myeloid leukemia is a rare malignancy with an average age of 70 years at diagnosis. Until recently, five-year survival of younger patients with this disease, despite being treated with allogenic hematopoietic stem cell transplantation, was < 30%, while in patients older than 60 years it was < 10%. **Treatment overview.** Due to the heterogeneity of acute myeloid leukemia no new drugs for treating this disease have been introduced for decades. The introduction of new drugs began from 2017: midostaurin, gilteritinib, CPX351, enasidenib, ivosidenib, venetoclax, glasdegib, while gemtuzumab ozogamicin has been reintroduced. Modern treatment strategies require an individual approach, based on prognostic parameters such as cytogenetical and molecular profile of acute myeloid leukemia at diagnosis and the assessment of minimal residual disease evaluated after two cycles of chemotherapy. Moreover, determining the eligibility of patients for ''intensive'' treatment, based on functional status, comorbidities and geriatric assessment of older patients, is necessary. Regarding the treatment of acute promyelocytic leukemia, the combination of arsenic trioxide and all-trans retinoic acid is universally accepted as the standard of care for non-high risk patients (WBC < $10x10^9$/L), while standard chemotherapy combined with all-trans retinoic acid is still used for high-risk patients (WBC >$10x10^9$/L). **Conclusion.** Novel therapeutic modalities, along with allo-HSCT have changed the outcome of AML patients. However, treating patients unfit for intensive chemotherapy, as well as patients with relapse/refractory disease, is still challenging.

Key words: Leukemia, Myeloid, Acute; Therapeutics; Prognosis; Risk Assessment; Treatment Outcome

Sažetak

Uvod. Akutna mijeloidna leukemija je redak malignitet čija je medijana uzrasta prilikom dijagnostikovanja 70 godina. Sve donedavno, petogodišnje preživljavanje mlađih pacijenata je, uprkos alogenoj transplantaciji matične ćelije hematopoeze bilo < 30% dok je kod pacijenata starijih od 60 godina bilo < 10%. **Savremeni terapijski pristup.** Decenijama nije bilo novih lekova za akutnu mijeloidnu leukemiju što je posledica izrazite heterogenosti ove bolesti. Od 2017. bivaju odobreni novi lekovi za akutnu mijelodnu leukemiju: midostaurin, gilteritinib, CPX351, enasidenib, ivosidenib, venetoclax i glasdegib, dok je ponovo odobrena upotreba gemtuzumab ozogamicina. Savremeno lečenje akutne mijelodne leukemije podrazumeva individualni pristup pacijentima koji se bazira na prognostičkim parametrima kao što su procena genetskomolekularnog rizika prilikom dijagnoze i minimalne rezidualne bolesti određene posle dva primljena ciklusa hemioterapije, sa jedne strane i procena podobnosti samog pacijenta za intenzivnu hemioterapiju na osnovu opšteg funkcionalnog stanja, komorbiditenog indeksa i gerijatrijske procene starijih od 60 godina, sa druge strane. U pogledu lečenja akutne promijelocitne leukemije, kombinacija arsen trioksida i all- trans-retinoične kiseline je prihvaćena kao optimalni tretman standardnog oblika bolesti (leukociti < $10x10^9$/L) dok se za lečenje oblika visokog rizika (leukociti >$10x10^9$/L) i dalje preporučuje kombinacija all-trans-retinoične kiseline i hemioterapije. **Zaključak.** Novi terapijski modaliteti, uz alogenu transplantaciju i uz individualni pristup pacijentu su značano izmeniti ishod akutne mijeloidne leukemije. Poseban izazov u lečenju predstavljaju pacijenti nepodobni za intezivnu hemioterapiju zbog starosti i/ili komorbiditeta i pacijenti sa relapsom/rezistentnom akutnom mijelodnom leukemijom.

Ključne reči: akutna mijeloidna leukemija; terapija; prognoza; procena rizika; ishod lečenja

Introduction

Acute myeloid leukemia (AML) is a rare malignancy with an estimated incidence of 5.06 patients per 100.000 people and with average age of 70 years at diagnosis [1]. Five-year survival of adult AML patients, treated with standard-dose chemotherapy (ChT) and allogenic hematopoietic stem cell transplantation (allo-HSCT), where indicated, was < 30% until recently. However, in patients older than 60 years, five-year survival was < 10% [2, 3]. Due to the striking biological and clinical heterogeneity of AML, no new drugs were introduced in treatment protocols for this disease until 2017. Risk stratification based on cytogenetical and mo-

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Abbreviations

FLT3	– fms-related receptor tyrosine kinase 3
FLT3-ITD	– mutation an internal tandem duplication (ITD)
FLT3-TKD	– mutation in the tyrosine kinase domain
Ara-C	– citarabine
IDH mutations	– mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2) genes
Bcl-2	– "B-cell lymphoma-2" cellular protein that inhibits apoptosis
OS	– overall survival
CR/CRi	– complete remission/incomplete remission
FDA	– The United States Food and Drug Administration
EMA	– European Medicines Agency
ELN	– European LeukemiaNet
DLI	– donor lymphocyte infusion

lecular profile of AML at diagnosis [4] and the assessment of minimal residual disease (MRD) [5], allow an individual approach to treatment and the identification of patients suitable for target therapy [6, 7].

Treatment Overview

The introduction of new drugs began from 2017 with: midostaurin - oral multikinase inhibitor registered for the treatment of de novo FLT3$^+$AML adults in addition to the standard-dose ChT; gilteritinib - selective oral AXL receptor kinase inhibitor approved for the treatment of adults with relapsed/refractory (R/R) FLT3-ITD$^+$ AML as a monotherapy; CPX351 - dual-drug liposomal encapsulation of ara-C and daunorubicin with the drug ratio of 5:1 which has been approved for the treatment of adults with therapy-related-AML (t-AML) and AML with myelodysplasia-related cytogenetic changes (MRC-AML); enasidenib and ivosidenib - oral selective inhibitors of mutated IDH1 and IDH2 enzymes approved as a monotherapy for adults with R/R AML; venetoclax - a highly selective Bcl-2 protein inhibitor approved in combination with either hypomethylating agents (HMA) or low-dose ara-C (LDAC) for the treatment of *de novo* AML patients aged ≥ 75 or those with comorbidities that preclude use of intensive induction ChT. Similarly, glasdegib, an oral hedgehog pathway inhibitor, combined with LDAC is approved for patients ≥75 years of age or those who are unfit for induction ChT. Additionally, gemtuzumab ozogamicin has been reintroduced as a treatment option for adults with de novo CD33$^+$ AML, as a monotherapy, or in combination with standard-dose ChT. Moreover, it can be used as a monotherapy for R/R CD33$^+$AML in children older than two years and adults [2]. Allo-HSCT is an essential treatment option for patients with high-risk cytogenetics and molecular profile and patients with measurable MRD after two cycles of high dose ChT [7].

FLT3 inhibitors

One of the most important advances in treatment of de novo FLT3+AML patients is the introduction of midostaurin. Namely, approximately 20% of AML patients express FLT3-ITD which has been associated with early relapse and short OS. Moreover, studies have reported that a higher mutant allelic burden is associated with a worse prognosis. On the other hand, around 10% of AML patients express FLT3-TKD mutation, whose prognostic significance is undetermined. Midostaurin was applied with standard-dose ChT ("3+7" induction, ≤ 4 cycles of consolidation) on days 8 to 21 of a 28-day treatment cycle and, afterward, as monotherapy over 12 months, reducing the rate of mortality by 22% (uncensored for allo-HSCT) compared to the control group, receiving placebo with ChT. Patients who underwent allo-HSCT in the first remission and who received midostaurin before transplantation, had longer remission duration and higher OS compared to the control group [7]. Gliteritinib, as a monotherapy, has been approved for the treatment of R/R FLT3-ITD$^+$ AMLin adults as well [9].

CPX351

CPX351 has been approved for treating adults with *de novo* t-AML and MRC-AML. Both aforementioned types of AML are associated with a very poor prognosis [10]. Results of the study including patients ≥ 60 years diagnosed with t-AML, MRC-AML and secondary AML showed that patients treated with CPX351 had significantly higher OS than those treated with standard-dose ChT (9.56 *vs.* 5.95 months). Moreover, the rate of mortality in patients treated with CPX351 aged 60-69 and 70-75 years was reduced by 32% and 45%, respectively [11].

mIDH inhibitors

Inhibitors of mutant IDH enzymes (mIDH) have contributed significantly to the treatment of AML. Enasidenib is an inhibitor of mIDH2 and ivosidenib is mIDH1 inhibitor. IDH enzyme mutations are present in 20% of AML patients. Namely, in the study which included adults with R/R AML, the use of enasidenib 100 mg daily led to a favorable response in 40% of patients lasting for 5.8 months on average. Additionally, in 19% of patients who achieved CR the duration of the response was 19.7 months (average) [12, 13].

Gemtuzumab-ozogamicin

Gemtuzumab-ozogamicin (GO) was re-approved for the treatment of CD33$^+$ AML. Namely, it was approved as a monotherapy for R/R AML in patients > 60 years in 2000. However, it was withdrawn from the market in 2010 due to adverse events. However, new studies have shown that GO, if administered in lower doses and in fractionated treatment regimens in addition to standard ChT, improves survival rates and prolongs CR, both in older patients with de novo AML and favorable/intermediate risk patients [14]. Therefore, its use is now approved for the treatment of adults with *de novo* CD33$^+$ AML, in combination with standard-dose ChT or as a monotherapy. Moreover, it could be used as a monotherapy in R/R CD33$^+$AML children aged > 2 years and adults [7].

Treatment of unsuitable patients

Novel drugs are also introduced for the treatment of elderly patients who are unsuitable for intensive ChT [15]. The eligibility of patients for ''intensive'' treatment is based on functional status, comorbidities and geriatric assessment of older patients with AML [7]. The current standard therapy for these patients are HMA - azacitidine and decitabine, with a remission rate of 26-28% [4]. FDA approved venetoclax combined with either HMA or LDAC as well as glasdegib with LDAC for the treatment of *de novo* AML patients ≥75 years, unsuitable for intensive ChT. Venetoclax, combined with LDAC or HMA provides CR or CRi in 62-68% of patients. Moreover, the average duration of remission is 11.3 months, while estimated one-year survival rate is 60-72%. The incidence of early death is 2-3% and response time is one month [16]. Glasdegib, combined with LDAC leads to significantly longer survival compared to patients treated with LDAC, only (8.8 *vs*. 4.9 months). Response rate (CR+CRi) was also markedly higher in the glasdegib group (20 *vs*. 4.5%) [17].

Relapsed/refractory AML

Almost 20% of younger and more than 50% of elderly patients do not achieve CR after at least two intensive induction regiments (primarily resistant). Additionally, relapse is registered among 50-70% of patients with AML. The prognosis of R/R AML is highly unfavorable and dependent mainly on the patient's suitability for intensive ChT and allo-HSCT. The 5-year OS for adults with AML after relapse is approximately 10% depending on prognostic factors (age, duration of first CR, cytogenetics at diagnosis, molecular features, and history of prior allo-HCT) [18]. Molecular re-evaluation at relapse is recommended in order to identify patients suitable for targeted salvage options. Thus, in case of relapse, it is advisable to repeat the analysis of the FLT3 gene mutations. Moreover, if the ivosidenib and enasidenib are available in presence of mIDH1/IDH2 should be determined [7]. Recent regulatory agency approval of several novel agents has transformed the treatment of R/R AML. If the AML relapse occurs after the allo-HSCT, especially after six months post-transplantation, secondary HSCT or DLI are recommended. For patients with R/R AML unsuitable for intensive ChT, HMA or LDAC combined with venetoclax, gilteritinib monotherapy for FLT3-ITD/FLT3-TKD positive patients, ivosidenib/enasidenib for mIDH1/IDH2 positive patients, melphalan or palliative/supportive care could be implemented [7].

Treatment of acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) is the most malignant form of AML and its treatment is different from other types of AML. The introduction of all-trans-retinoic acid (ATRA) and later arsenic trioxide (ATO) made a revolution in the treatment course and prognosis of APL [19, 20]. Precisely, ATRA induces maturation of APL blasts through a conformational change of the PML–RARA fusion transcripts. This leads to terminal myeloid differentiation of APL blasts and apoptosis [21]. Firstly, ATRA was used as monotherapy in 1987 with excellent results regarding CR achievement. However, relapse rate remained high despite continuous ATRA treatment. That is why anthracyclines with or without cytarabine were reintroduced in APL treatment simultaneously with ATRA. This combination became the standard of care for APL in the nineties [19]. However, the mechanism of action of ATO has not been fully understood. It has been shown that it induces both differentiation and apoptosis of APL blasts [21]. In 2000 ATO was approved as a monotherapy for R/R APL [19]. Later, studies regarding ATO therapy in newly-diagnosed APL patients with or without ATRA were launched. Results of the trials APL0406 and AML17 have shown lower relapse rate and better OS in „ATRA+ATO" arm compared to „ATRA+ChT" arm provided that „ATRA+ATO" (CT-free regimens) has become the standard of care for patients with standard-risk APL [21–23]. For, patients with high-risk APL, optimal treatment is still the subject of debate. According to the latest ELN recommendations, ATO+ATRA could be used in high-risk patients along with idarubicin and GO [24]. However, ATO and GO are not approved by EMA for high risk APL and, for that reason, „ATRA+ChT" is still the standard of care for this group of patients [25, 26].

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THE CURRENT APPROACH AND THE TREATMENT OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

SAVREMENI PRISTUP I LEČENJE ADOLESCENATA I MLADIH ODRASLIH SA AKUTNOM LIMFOBLASTNOM LEUKEMIJOM

Borivoj SEKULIĆ

Summary

Introduction. The treatment outcome of adolescents and young adults with acute lymphoblastic leukemia is much poorer in contrast to pediatric patients. By changing the concept of the treatment for patients who are adolescents and young adults with acute lymphoblastic leukemia, especially with the use of pediatric regimens, significant improvement in survival has been made (current 5-year survival rate goes up to 70%). **Contributing factors for different outcomes between children and adolescents and young adults with acute lymphoblastic leukemia.** Beside the differences between pediatric and adult protocols, there are several factors which can explain the different outcomes between these groups of patients with acute lymphoblastic leukemia. One of the main factors is different biology of the leukemias and, on the other side, lower accrual rates in clinical trials in adolescents and young adults and their specific psychosocial factors, like poor compliance with the treatment and missed appointments. **Current treatment and novel approaches in the treatment of adolescents and young adults with acute lymphoblastic leukemia.** Current treatment approach to the adolescent and young adults with acute lymphoblastic leukemia is based on the pediatric protocols with the risk-adapted strategy, which depends primarily on the cytogenetics and postinduction minimal/measurable residual disease. The main goal of the novel treatment, especially with the use of targeted therapy and innovative immunotherapies incorporated in the pediatric protocols, is to achieve a deep and durable leukemia-free survival. To transplant or not to transplant adolescents and young adults with acute lymphoblastic leukemia is still a matter of debate, particularly in the era of pediatric regimens and the new sequence algorithm with the upfront use of novel drugs. **Conclusion.** Adolescent and young adult patients with acute lymphoblastic leukemia should be treated in specialized centers by an experienced multidisciplinary team with close attention to their particular needs.

Key words: Precursor Cell Lymphoblastic Leukemia-Lymphoma; Diagnosis; Therapeutics; Adolescent; Young Adult; Treatment Outcome; Pediatrics; Clinical Protocols

Sažetak

Uvod. Ishod lečenja adolescenata i mladih odraslih bolesnika sa akutnom limfoblastnom leukemijom je znatno lošiji u odnosu na pedijatrijske bolesnike. Promenom koncepta lečenja adolescenata i mladih odraslih sa akutnom limfoblastnom leukemijom, posebno uvođenjem pedijatrijskih protokola u lečenje, značajno je poboljšano preživljavanje u ovoj grupi bolesnika (aktuelno 5-godišinje preživljavanje ide i do 70%). **Faktori koji doprinose različitom ishodu lečenja dece i adolescenata i mladih odraslih bolesnika sa akutnom limfoblastnom leukemijom.** Pored razlika u pedijatrijskim i adultnim protokolima koji se koriste u lečenju akutne limfoblastne leukemije, postoji još nekoliko faktora koji bi mogli objasniti razliku u ishodu lečenja između ove dve grupe bolesnika. Jedan od glavnih faktora je različita biologija leukemija, a sa druge strane i niža stopa uključivanja adolescenata i mladih odraslih bolesnika u kliničke studije i njihovi specifični psihosocijalni faktori, kao što su loša komplijansa i nedolaženje na redovne kontrole. **Aktuelno lečenje i novi pristupi u lečenju adolescenata i mladih odraslih bolesnika sa akutnom limfoblastnom leukemijom.** Savremeno lečenje ovih bolesnika se bazira na upotrebi pedijatrijskih protokola i vodi se u odnosu na rizik, koji se temelji pre svega na citogenetici i postindukcionoj minimalnoj/merljivoj rezidualnoj bolesti. Inovativni pristup lečenju ove grupe bolesnika, posebno sa upotrebom ciljane i nove imunoterapije koja je inkorporirana u pedijatrijske protokole, ima za cilj postizanje dubokog i trajnog odgovora. I dalje ostaje otvoreno pitanje da li transplantirati ove bolesnike, posebno u eri primene intenzivnih pedijatrijskih protokola i inovativnih lekova, koji se pomeraju u prvu liniju terapije. **Zaključak**. Adolescenti i mladi odrasli sa akutnom limfoblastnom leukemijom zaslužuju da se leče u specijalizovanim centrima i da u njihovo lečenje budu uključeni multidisciplinarni timovi, koji će posebnu pažnju posvetiti njihovim specijalnim potrebama.

Ključne reči: akutna limfoblastna leukemija; dijagnoza; terapija; adolescenti; mladi; ishod lečenja; pedijatrija; klinički protokoli

Introduction

In contrast to acute lymphoblastic leukemia (ALL) in children with 5-year survival rates of 90%, the outcome of the adult patients with ALL is much poorer, with overall survival rates of only 30%-40%. A unique group of patients with ALL are adolescents and young adults (AYA), accounting for 20-25% of newly diagnosed ALL patients. It is not only the age category, but a subset of patients with special biology of the ALL and special needs. In the last two decades, by changing the concept and ap-

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Abbreviations

AYA	– adolescents and young adults
ALL	– acute lymphoblastic leukemia
ETV6-RUNX1	– ETS Variant Transcription Factor 6-Runt-related transcription factor 1
Ph	– Philadelphia chromosome
ETP-ALL	– early T precursor acute lymphoblastic leukemia
MRD	– minimal/measurable residual disease
CNS	– central nervous system
CR1	– first complete remission
HyperCVAD	– hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone
BFM	– Berlin-Frankfurt-Münster
CALGB	– Cancer and Leukemia Group B
NOPHO	– Nordic Society of Pediatric Hematology and Oncology
NCCN	– National Comprehensive Cancer Network
TKI	– Tyrosine Kinase Inhibitors
SCT	– stem cell transplantation
ABL	– Abelson Family of Tyrosine Kinases
PDGFRB	– Platelet Derived Growth Factor Receptor Beta
JAK2	– Janus Kinase 2
JAK-STAT	– Janus Kinase- Signal Transducer and Activator of Transcription
CD	– cluster of differentiation
CAR T	– chimeric antigen receptor T cell
NCT	– National Clinical Trials

proach to the treatment, significant improvement in survival of AYA patients with ALL has been made (5-y survival rates of 30–45% with traditional adult regimens, has dramatically increased to 60–70%, using pediatric regimens) [1].

Contributing factors for different outcome between children and AYA with ALL

There are different age limits used for defining AYA patients. While most of the physicians agree that adolescents and young adults begin from the age of 15 to 18, there is not a uniquely accepted upper limit, which differs depending on different parts of the world. The arbitrary upper limit age for AYA in Europe is 24, in contrast to America, where the National Cancer Institute has broadened ranges for this population of cancer patients to 39 years old [2]. AYA patients with ALL were previously treated with traditional adult regimens, but the outcomes were worse comparing with the treatment of children with ALL. There are several possible explanations for this finding. One is the difference in the biology of ALL between adults and AYA, then differences in pediatric *versus* adult regimens, but also lower accrual rates in clinical trials in this age population and some psychosocial factors, like poor compliance with treatment and missed appointments. Unlike children, AYA patients have ALL with lower incidence of good prognostic cytogenetic and molecular markers (like t(12;21)/ETV6-RUNX1, as well as hyperdiploidy). On the other side, there is an increased prevalence of resistant ALL subtypes in AYA population, like Philadelphia chromosome positive ALL (Ph+ ALL), Philadelphia chromosome like ALL (Ph-like ALL), ALL with t(4;11) and others [3]. There is also a higher incidence of T-cell ALL among AYA patients in contrast to children, especially the high risk early T precursor (ETP) ALL. Differences in biology of ALL have profound reflections on the sensitivity to the treatment, which explains deeper responses (complete remissions with negative minimal residual/measurable disease (MRD)) to induction treatment in children with ALL and therefore better outcome when compared to AYA patients. According to the study by Stock and colleagues, only 44% AYA patients treated with a pediatric regimen became MRD negative [4]. Pediatric regimens used to treat children with ALL are usually more demanding, more intensive and toxic, and differ markedly from adult regimens in several issues. They consist of more cumulative doses of glucorticoids and vincristin, higher doses of methotrexate as well, and apsaraginase is an essential part of the treatment. Pediatric protocols, as a rule, include late intensification and early and intensive central nervous system (CNS) prophylaxis. Conversely, adult protocols use more myelosuppressive drugs (like anthracyclines, cyclophosphamides…) and more patients (about 30% of AYA) are transplanted in the first complete remission (CR1), in contrast to less than 5–10% of children in CR1 [5]. Considering this, numerous retrospective, as well as prospective clinical trials were conducted and confirmed the eligibility and improved outcome with the use of pediatric regimens in treating AYA patients [6]. There are some trials, which did not confirm superiority of pediatric regimens over adult protocols in terms of improved survival. One of them is a monocentric study from MD Anderson Cancer Center, which found similar 5-y overall survival rates (around 60%) in both groups of AYA patients, treated with Hyper CVAD and those treated with augmented BFM (Berlin-Frankfurt-Münster) pediatric protocol [7]. One of the largest prospective clinical trials, which showed advantage and feasibility of intensive pediatric regimens in treating AYA patients up to 40 years old, is the American intercooperative group trial (CALGB 10403). This trial enrolled about 300 patients, 89% achieved CR, treatment related mortality was relatively low (3% during induction) and 3-y overall survival was 73% [4]. Similar results with the use of pediatric regimens for AYA patients were reported from the Japan Adult Leukemia Study Group and Nordic group (NOPHO) [8, 9]. However, more intensive treatment and the wider use of asparaginase in AYA patients are related to the higher rates of specific complications, like liver toxicities, pancreatitis and thrombosis, especially during induction treatment [10]. Other factors contributing to the difference in outcomes between children and AYA patients with ALL are psychosocial factors and lower accrual rates in clinical trials. Poor treatment compliance, especially during maintenance, is higher in AYA

group of patients and has been associated with two to threefold increase in the risk of relapse [11]. AYA patients treated out of clinical trials had decreased survival rate by 20% when compared with patients enrolled in clinical trials [12]. It is recommended to treat AYA patients in specialized, tertiary care centers, which are able to provide access to clinical trials and innovative drugs, and with multidisciplinary approach and full psychosocial support.

Current treatment and novel approaches in the treatment of AYA patients with ALL

Use of pediatric regimens in the treatment of AYA patients is widely accepted in most countries, according to a recent investigation in Australia, more than 80% of AYA patients have been treated with pediatric protocols [13]. There is not only one, universally accepted, pediatric regimen, because there are no randomized comparative trials, which could prioritize one protocol over another. National Comprehensive Cancer Network (NCCN) guidelines emphasize that, beside clinical trial, the pediatric-inspired regimens are "preferred" for all forms of Ph-negative ALL, but that multi-agent chemotherapy is also an option [14]. The risk adapted treatment approach is generally accepted and the risk stratification is based primarily on cytogenetics and minimal/measurable residual disease (MRD). New immunotherapeutic drugs (blinatumomab and inotuzumab ozogamicin), with a proven efficacy in relapsed/refractory patients with ALL, are currently investigated as a part of frontline protocols in order to induce deeper remissions [5].

In the initial workup of the AYA patients with ALL it is especially important to identify ALL subtypes with prognostic significance and potential targeted treatment, like Ph+ ALL, Ph-like ALL and early T precursor (ETP) ALL. The Philadelphia chromosome occurs in around 20% of AYA patients with ALL. Current guidelines for the treatment of Ph+ ALL recommend, in the induction phase, use of tyrosine kinase inhibitors (TKI) in combination with chemotherapy or TKI in combination with corticosteroid [14]. Several studies showed that TKI in combination with less intensive chemotherapy, or even chemotherapy-free regimens, are equally effective as the combination of TKI with intensive chemotherapy, with a higher CR rate and fewer deaths in induction [15]. Consolidation treatment is usually based on chemotherapy plus TKI or stem cell transplantation. Stem cell transplantation (SCT) in CR1, with post-transplant TKI maintenance, is still the standard of care in eligible adult patients with Ph+ ALL, even though use of newer generation TKIs in combination with chemotherapy or second/third generation TKI with post-induction blinatumomab could change the current SCT algorithm [16, 17]. Ph-like ALL is more common subtype of ALL in AYA population (up to 30%) than in other age categories. A typical patient with Ph-like ALL is a young male patient with hyperleucocytosis and a poorer response to induction treatment, as well as a dismal prognosis. In this subtype of ALL, many kinases (ABL, PDGFRB, JAK2, JAK-STAT,…) can be activated, which further provide proliferative advantage to leukemic cells, but on the other side they also can be targets for specific treatment with TKIs or JAK2 inhibitors. There is no standard diagnostic and therapeutic approach to these patients, so it is recommended to enroll patients with Ph-like ALL in a clinical trial or to use innovative drug (blinatumomab) to eradicate MRD and proceed with an allogeneic transplant [3]. ETP ALL is relatively rare (around 15% of T ALL), with a characteristic immunophenotype: $CD1a^-$, $CD8^-$, $CD5^-$ (dim) and positivity for one or more stem cell or myeloid antigens and poor response to treatment. There is no specific treatment for this high risk T ALL subgroup, novel treatment strategies are needed and early referral to transplantation is highly recommended [18].

A question that arises in the post-remission treatment of AYA patients is whether or not to transplant in CR1. The current approach to the treatment of AYA patients, with the use of intensive pediatric regimens and novel immunotherapy, causes a deeper and more durable response. On the other side, there are significant early (treatment-related mortality from 10-30%) and late toxicities related to stem cell transplantation in this vulnerable age category. Seftel and colleagues showed in a retrospective comparison trial that younger adults with Ph negative ALL treated with pediatric inspired chemotherapy had superior overall survival compared to allogeneic transplantation [19]. All things considered, allogeneic stem cell transplantation is reserved only for AYA patients with ALL in CR1 with suboptimal early response to treatment, essentially based on MRD and with high risk cytogenetics and molecular profile [20]. The on-going CASSIOPEIA trial (NCT03876769) should answer whether the new immunotherapy (chimeric antigen receptor (CAR) T-cell therapy) has the potential to replace SCT in the treatment of AYA patients with high risk ALL, who are MRD positive at the end of consolidation phase of the treatment.

Conclusion

Wider use of pediatric regimens in combination with the novel immunotherapy, as well as a risk/MRD-adapted approach significantly improved the outcome of AYA patients with ALL. The novel concept of treatment for these patients, using targeted therapies and early use of modern immunotherapies incorporated in a backbone of pediatric chemotherapy, ultimately aims to achieve durable leukemia-free survival and to avoid the need for an allogeneic transplantation. AYA patients with ALL should be treated in specialized centers by an experienced multidisciplinary team with close attention to their unique needs.

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TREATMENT OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA WHO ARE NOT SUITABLE FOR HIGH-DOSE CHEMOTHERAPY AND HEMATOPOIETIC STEM CELL TRANSPLANTATION

LEČENJE BOLESNIKA SA AKUTNOM LIMFOBLASTNOM LEUKEMIJOM KOJI NISU POGODNI ZA VISOKODOZNU HEMIOTERAPIJU I TRANSPLANTACIJU MATIČNE ĆELIJE HEMATOPOEZE

Nenad GOVEDAROVIĆ

Summary

Introduction. Acute lymphoblastic leukemia is a malignant disease characterized by the proliferation of precursor B-cells, T-cells or less often, precursors of NK-cells. B-cell acute lymphoblastic leukemia is more common in patients >60 years of age compared to patients <60 years of age (89% vs. 66%), and cytogenetic abnormalities such as t(9;22) (Ph+) are more common in older than younger patients (36% against 19%). Elderly patients often have a poor status and comorbidities, so poor disease outcome is more common. **Clinical and biological features.** B cell acute lymphoblastic leukemia is more common in patients >60 years of age compared to patients <60 years of age (89% vs 66%) and cytogenetic abnormalities such as t(9;22) are more common in older vs. younger (36% vs. 19%). **Therapy.** The elderly and patients with comorbidities require less intensive therapy, based on corticosteroids, vincristine and asparaginase, while avoiding anthracyclines and alkylating agents, due to the high mortality associated with treatment. For "unfit" patients with Ph-positive acute lymphoblastic leukemia, tyrosine kinase inhibitors with reduced-intensity chemotherapy or corticosteroids alone are recommended. For t(9;22) negative patients, low-dose corticosteroid chemotherapy with or without immunotherapy is recommended. For patients with T-cell acute lymphoblastic leukemia, chemotherapy with venetoclax may be an option. **Conclusion.** The introduction of targeted therapy has changed treatment options in acute lymphoblastic leukemia. For elderly patients, targeted therapy is a necessary modality since standard chemotherapy leads to a poor outcome due to its toxicity and ineffectiveness.

Key words: Precursor Cell Lymphoblastic Leukemia-Lymphoma; Treatment Outcome; Risk Factors; Aged; Diagnosis; Therapeutics; Comorbidity

Sažetak

Uvod. Akutna limfoblastna leukemija je maligna bolest koja se karakteriše proliferacijom prekursorskih B-ćelija, T-ćelija ili ređe, prekursora NK ćelija. B-ćelijska akutna limfoblastna leukemija je češća kod pacijenata mlađih od 60 godina u poređenju sa pacijentima starijim od 60 godina (89% prema 66%), a citogenetske abnormalnosti kao što je t(9;22) češće su kod starijih nego kod mlađih pacijenata (36% prema 19%). Stariji pacijenti često imaju loš status i komorbiditete, pa je loš ishod bolesti češći. **Kliničke i biološke karakteristike.** B-ćelijska akutna limfoblastna leukemija je češća kod pacijenata mlađih od 60 godina u poređenju sa pacijentima starijim od 60 godina (89% prema 66%) i citogenetske abnormalnosti kao što je t(9;22) češće su kod starijih u odnosu na mlađe (36% prema 19%). **Terapija.** Stariji i bolesnici sa komorbiditetima zahtevaju manje intenzivnu terapiju, zasnovanu na kortikosteroidima, vinkristinu i asparaginazi, uz izbegavanje antraciklina i alkilirajućih agenasa zbog visoke smrtnosti u vezi sa lečenjem. Za *unfit* pacijente sa Ph pozitivnom akutnom limfoblastnom leukemijom, preporučuju se inhibitori tirozin kinaze sa hemoterapijom smanjenog intenziteta ili samo kortikosteroidima. Za t(9;22) negativne pacijente, preporučuje se niskodozna hemoterapija sa kortikoidima sa imunoterapijom ili bez nje. Za pacijente sa T-ćelijskom akutnom limfoblastnom leukemijom hemioterapija sa venetoklaksom može biti opcija. **Zaključak.** Uvođenje ciljane terapije promenilo je opcije lečenja u akutnoj limfoblastnoj leukemiji. Starijim pacijentima je ciljana terapija neophodan modalitet budući da standardna hemoterapija zbog svoje toksičnosti i neefikasnosti dovodi do lošeg ishoda.

Ključne reči: akutna limfoblastna leukemija; ishod lečenja; faktori rizika; stari ljudi; dijagnoza; terapija; komorbiditet

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized on the proliferation of B-cell, T-cell precursors, or rarely NK-cell precursor. The disease has a bimodal incidence, with a peak in younger <20 years (54.2%), whereas approximately 20% cases occur in those aged above 55 year. Overall incidence of ALL in Europe is 1.28/100.000 annually. As there is an increasing proportion of the elderly population in Europe, an increase in the incidence of ALL in this age group is to be expected [1].

Clinical and biological features

B-lineage ALL is more frequent in patients >60 years) compared to patients < 60 years (89% vs. 66%) and the cytogenetic abnormalities such as t(9;22) (Ph+) are more common in older than young-

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Abbreviations

ALL – Acute lymhoblastic leukemia
ADC – Antibody (anti CD 22 + calicheamicin) Drug Conjugate
BITE – bispecific anti CD3/anti CD19 monoclonal antibody
CD – Cluster of differentiation
CR – complete remission
HSCT – Hematopoietic Stem Cell Transplantation
MRD – minimal residual disease
MTX – metotrexate
POMP – Purinethol, Oncovin, Metotrexate, Prednisolone;
R/R – relapsed/refractory disease
TKI – Tirosin kinase inhibitors

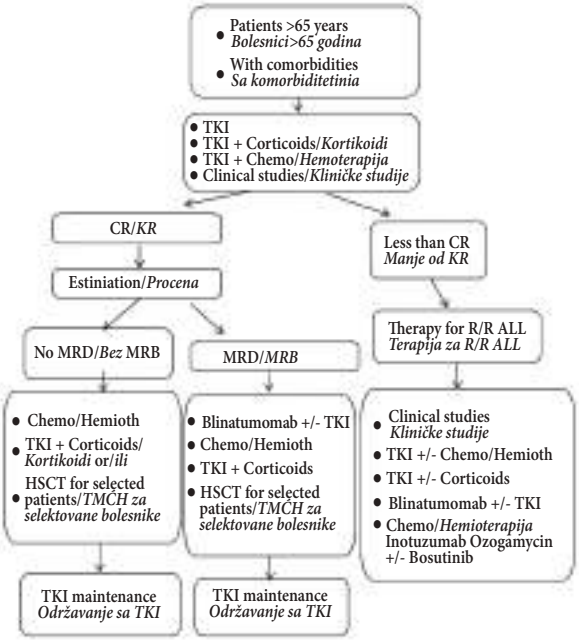
er patients (36% vs. 19%). From the clinical aspect, bulky disease, high white blood cell count, or rapid progression in older patients is less common. At diagnosis, up to 43% of patients older than 60 years have a performance status of more than 2 [1].

Diagnosis

Bone marrow puncture is mandatory, whereby the finding >20% of blasts supports the diagnosis of ALL. Blasts are atypical lymphoid or undifferentiated cells. The immunophenotyping by flow citometry has a key diagnostic role, demonstrating commitment of the blast cell to the B- or T-cell lineage [2]. A further diagnostic step is molecular screening for BCR-ABL1 gene rearrangement by fluorescence in situ hybridisation (FISH), or translocation t(9;22) (q34;q11), typical of Ph+ ALL, which is sensitive to treatment with tyrosine kinase inhibitors (TKI) [3].

Approach to therapy of unfit patients with ALL

Older patients often have a poor performance status and comorbidities and therefore a poor outcome is more common. The most frequent comorbidities are diabetes (46%), vasculopathia (18%), cardiac failure (15%) and pulmonary disease (12%). In addition, bad compliance, depression or dementia, may of issue during treatment [1, 4]. Factors related to the patient



Scheme 1. Therapy algorithm for unfit patients with BCR/ABL+ ALL (NCCN, 2021)

Shema 1. *Terapijski algoritam za nepodesne bolesnike sa BCR/ABL+ ALL (NCCN, 2021)*

Legend: ALL – Acute lymhoblastic leukemia; TKI – Tyrosine kinase inhibitors; HSCT– Hematopoietic Stem Cell Transplantation; CR – complete remission; MRD – minimal residual disease; R/R – relapsed/refractory disease.

Legenda: ALL – Akutna limfoblastna leukemija; TKI – Tirozin kinaza inhibitor; TMĆH– Transplantacija matične ćelije hematopoeze; KR – kompletna remisija; MRB – minimalna residualna bolest; R/R – relaps/refraktarna bolest.

and the disease should be considered when starting therapy. In the use of the indexes for assessing the performance status of the patient (ECOG), the Charlson comorbidity index is widely accepted [1, 5], while

Table 1. Targeted therapy for unfit patients with BCR/ABL+ Acute lymhoblastic leukemia

Tabela 1. *Ciljana terapija za nepodesne bolesnike sa BCR/ABL+ akutnom limfoblastnom leukemijom*

Name/*Ime*	Type/*Tip*	Recommended dose/*Preporučena doza*	Source/*Izvor*
Imatinib	TKI	600 mg p.o. daily/*dnevno*	NCCN 2021
Imatinib + corticoids/*kortikoidi*	TKI	800 mg p.o. D1 – D45 + Pronison 45 mg /m2 p.o. D1-D45	GIMEMA LAL 0201-B
Dasatinib + corticoids/*kortikoidi*	TKI	140 mg p.o. D1 – D7 + Dexason 40 mg i.v. D1 –D7	CALGB 10701
Inotuzumab ozogamicin	ADC	Inotuzumab + Ozogamycin weekly doses 1.8 mg/m^2 + i.t. prophylaxis monthly/*i.t. profilaksa mesečno*	NCCN 2021
Ponatinib	TKI	45 mg p.o. 48 weeks/nedelja + Pronison 60mg/m2 p.o. D14-D29 i.t. prophylaxis monthly/*i.t. profilaksa mesečno*	INCB84344-201 GIMEMA LAL 1811
Blinatumomab	BITE	28 mcg i.v. D1-D28	NCCN 2021

Legend: MTX – metotrexate; TKI – Tirosin kinase inhibitor; ADC – Antibody Drug Conjugate; BITE – bispecific anti CD3/anti CD19 monoclonal antibody

Legenda: MTX – metotreksat; TKI – Tirozin kinaza inhibitori; ALK – Antitelo - lek - konjugat; BITE – bispecifično anti CD3/anti CD19 monoklonsko antitelo

Table 2. Therapy with Tyrosine kinase inhibitors with low intensity chemotherapy for unfit patients with BCR/ABL+ Acute lymhoblastic leukemia
Tabela 2. *Terapija sa inhibitorima tirozin kinaze i hemoterapijom niskog intenziteta za nepodesne bolesnike sa BCR/ABL+ akutnom limfoblastnom leukemijom*

Drug/*Lek*	Doses/*Doze*	Source/*Izvor*
Dasatinib + chemo *hemoterapija*	Pre-phase/*Prefaza*: Dexamethasone 10 mg daily/*dnevno*, from/*od* D-7 to/*do* D-3 Induction/*Uvodna terapija*: D1 MTX i.t. 15 mg Dasatinib 140 mg p.o. daily/*dnevno* Vincristine 2 mg i.v. weekly/*nedeljno* Dexamethasone 40 mg, 2 days during 4-weeks cycle *2 dana tokom 4-nedeljnog ciklusa.* Consolidation/*Konsolidacija*: Dasatinib 100 mg p.o. daily/*dnevno* + MTX 1000 mg/m^2 i.v. D1 L-asparaginase 10000 U/I i.m. D2 (cycles/*ciklusi* 1,3,5) ARA-C 1000 mg/m^2 i.v./12h D1, D3, D5 (cycles/*ciklusi* 2,4,6), in 4-week intervals/ u *4-nedeljnim intervalima*. Maintenance therapy/*Terapija održavanja* (2-3 months/*meseca*) Dasatinib 100 mg p.o. daily/*dnevno* 6-MP (Purinethol) 60 mg/m^2 p.o. daily/*dnevno* 5-7 days/*dana* MTX 25 mg/m^2 p.o. weekly/*nedeljno* Post-maintenance/*Terapija nakon terapije održavanja*: Dasatinib 100 mg p.o. daily/*dnevno*	EWALL-Ph-01
Nilotinib + chemo *hemoterapija*	Pre-phase/*Prefaza*: Dexametasone 10 mg/m^2 from/*počev* od D-7 to/*do* D-3 Induction therapy/*Indukcija*: Nilotinib 2 x 400 mg p.o. continiously/*kontinuirano* Vincristine 1 mg i.v. Dexason 40 mg i.v. 2 days during 4- weeks cycle *2 dana tokom 4-nedeljnog ciklusa* Consolidacija/*Konsolidacija*: Nilotinib a 400 mg 2 x 1 p.o. MTX 1000 mg/m^2 i.v. D1 L-Asparaginase 10000 U/m^2 (cycles/*ciklusi* 1,3,5) ARA-C 1000 mg/m^2 i.v./ 12h, D1, D3, D5 (cycles/*ciklusi* 2,4,6) Maintenance therapy/*Terapija održavanja*: (24 months/*24 meseca*) Nilotinib 2x400 mg p.o. + 6-MP (Purinethol) 4 x 1 MtX 25mg/m^2, weekly/*nedeljno*	EWALL-Ph-02

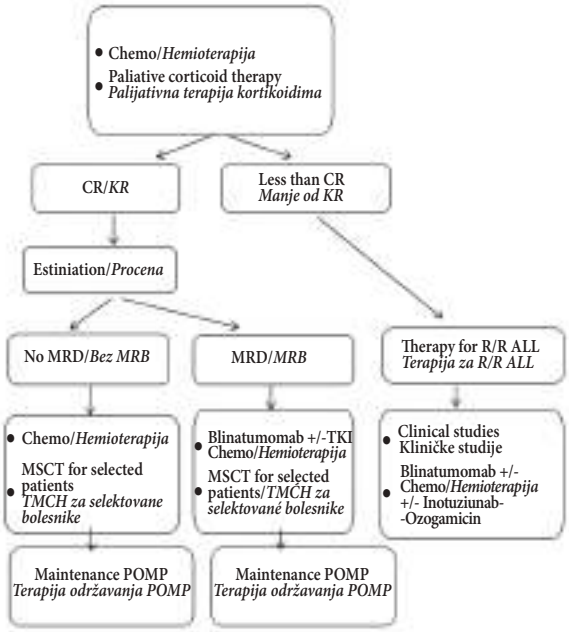
the HCT-CI index is used for patients who are candidates for allogeneic HSC transplantation [6].

In Europe, 5-year overall survival (OS) is about 41%. Thus, in a group of 15–54 years, OS was >50%, in a group from 55-64 years OS was <30%, and in patients over 65 years, OS was <20% [3]. Patients with precursor ALL have shorter survival and poor prognosis, especially those with minimal residual disease (MDR) after therapy. MDR indicates the presence of leukemic blasts below the di-

Table 3. Low intensity chemotherapy for unfit patients with BCR/ABL negative Acute lymhoblastic leukemia
Tabela 3. *Terapija niskog intenziteta za bolesnike sa BCR/ABL negativnom akutnom limfoblastnom leukemijom*

Mini CVD	Recommended doses/*Preporučene doze*	Source/*Izvor*
Cycles/*Ciklusi* 1,3,5,7	Cyclophosphamide 150 mg/m^2/12h D1-D3 Dexamethasone 20 mg p.o. ili i.v. D1-D4 D11-D14 + Vincristine a 2 mg i.v. D1, D8	NCT01371630
Cycles/*Ciklusi* 2,4,6,8	MTX 250 mg/m^2 i.v./ARA-C 0·5 g/m^2/12 h D2, D3	
+/ -	Inotuzumab ozogamicin C1D3 1,8 mg/m^2 C2D3 C3D3, C4D3 1,3 mg/m^2	
POMP maintenance therapy *Terapija održavanja* 1–3 years/*godine*	Purinethol a 50 mg p.o., 3 x 1, D1-D28 Vincristin a 2 mg i.v. D1 MTX a p.o. 1x weekly/*nedeljno* Prednisone 200 mg p.o. D1 – D4	

* For patients with CD20$^+$ > 20% ALL, Rituximab has to be administered in cycles 1,2,3,4
** Pacijentima sa CD20$^+$ >2 0% ALL, Rituksmab treba dati u ciklusima 1,2,3,4*



Scheme 2. Therapy algorithm for unfit patients with BCR/ABL negative ALL (NCCN, 2021)
Shema 2. Terapijski algoritam za nepodesne bolesnike sa BCR/ABL negativnom ALL (NCCN, 2021)
Legend: ALL – Acute lymhoblastic leukemia; HSCT– Hematopoietic Stem Cell Transplantation; CR – complete remission; MRD – minimal residual disease; R/R – relapsed/refractory disease. POMP – Purinethol, Oncovin, Metotrexate, Prednisolone
Legenda: ALL – Akutna limfoblastna leukemija; TMĆH – Transplantacija Matične ćelije Hematopoeze; KR – kompletna remisija; MRB – minimalna rezidualna boleste; R/R – relapsirajuća ili refraktarna bolest; POMP – Purinethol, Oncovin, Metotreksat, Prednizolon

agnostic threshold using standard morphological methods. Assessment of MRD status is crucial for treatment decision, and the purpose of therapy is to achieve MRD-negative status in the early phase of ALL treatment [3, 7]. There is no strict consensus for "unfit" patient, but the term mainly refers to patients older than 55 – 65 years, frail patients > 70/75 years (unsuitable for any chemotherapy), with poor performance status (ECOG>2), with Charlson comorbidity index (CCI) ≥ 5 and those with HCT-CI index ≥ 3.

Therapy

Generally, unfit patients demand less intensive therapy, based on corticosteroids, vincristine and asparaginase, avoiding anthracyclines and alkylating agents, because of treatment-related mortality. In 2021., the National Comprehensive Cancer Network (NCCN) issued guidelines for the treatment of the ALL Ph+ unfit patients [8, 9], which is shown in **Scheme 1**.

For unfit patients with Ph-positive ALL, TKI or TKI with reduced-intensity, chemotherapy or corticosteroids alone are recommended. *Ponatinib,* third generation TKI, can overcome several resistance mechanisms in patients previously treated with Ph+ ALL. Nevertheless, relapses are a regular occurrence, and therapy with monoclonal antibodies was developed, which is more tolerable for unfit patients [9]. *Blinatumumab,* a bi-specific antibody which involves engagement of the patient's T-cells with CD19-expressing tumor cells, is indicated as monotherapy for the treatment of CD19 positive relapsed/refractory B-precursor ALL [10]. For unfit BCR/ABL+ ALL patients the option is a combination of TKI with low intensity chemotherapy, which is shown in a **Table 2**.

For patients with BCR/ABL negative ALL, is the option is a combination of reduced dose hyper CVAD, also known as mini CVD [9, 10], as seen in **Table 3**.

According to NCCN guidelines, a therapy algorithm exists for unfit patients with BCR/ABL negative ALL, as seen on a **Scheme 2**.

Therapy of unfit patients with T ALL

In 2021., MD Anderson developed an algorithm for the treatment of unfit patients with T ALL. For these patients mini-CVD regimen + Venetoclax (BCL-2 inhibitor) is recommended. Patients who enter complete remission have to be considered for consolidation/maintenance therapy. For others, inclusion in clinical studies or salvage therapy is recommended.

Conclusion

The introduction of targeted therapy has changed the treatment options in acute lymhoblastic leukemia. Elderly patients are in need of novel therapies because standard chemotherapy leads to poor outcome. High remission rates with tirosin kinase inhibitors therapy, with or without low doses of chemo, make it the therapy of choice for Ph positive, unfit patients with acute lymhoblastic leukemia. For Ph negative acute lymhoblastic leukemia patients, low dose chemotherapy with corticoids +/- immunotherapy is recommended. For patients with T acute lymhoblastic leukemia, low dose chemotherapy with venetoclax, could be an option.

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MYELODYSPLASTIC SYNDROME
MIJELODISPLASTIČNI SINDROM

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DIAGNOSIS, CLASSIFICATION, AND PROGNOSIS OF MYELODYSPLASTIC SYNDROMES

DIJAGNOZA, KLASIFIKACIJA I PROGNOZA MIJELODISPLASTIČNOG SINDROMA

Andrija BOGDANOVIĆ[1, 2], Dragomir MARISAVLJEVIĆ[3] and Aleksandar SAVIĆ[4, 5]

Summary

Introduction. Myelodysplastic syndromes represent clonal neoplastic disorders characterized by hematological dysplasia, ineffective hematopoiesis, cytopenia, and increased risk of transformation to acute myeloid leukemia. **Material and Methods.** A literature review was conducted using the following bibliographic databases: Google Scholar, MEDLINE, and Kobson. The recommendations for diagnosis, classification, and prognosis are based on expert opinions grounded on a review of the literature and contemporary recommendations for diagnosis and prognosis in myelodysplastic syndrome. **Diagnosis and classification.** Diagnosis of myelodysplastic syndrome should be based on detailed patient and family history, physical examination, and comprehensive blood examinations in to exclude all other causes of cytopenia and dysplasia. Mandatory for myelodysplastic syndrome diagnosis is cytology of blood and bone marrow, bone marrow biopsy with immunohistology and cytogenetics. 2016 World Health Organization classification should be used for myelodysplastic syndrome diagnosis. SF1B3 genetic analysis is recommended in patients with suspected myelodysplastic syndrome with ringed sideroblasts and p53 mutation status. **Prognosis**. Revised International Prognostic Scoring System for myelodysplastic syndrome (IPSS-R) risk score should be defined for every patient in order to determine prognosis. The next-generation sequencing could provide additional diagnostic and prognostic information, particularly in young transplant candidates. **Conclusion.** Myelodysplastic syndrome diagnosis is based on the 2016 World Health Organization classification. The prognosis should be based on the Revised International Prognostic Scoring System with the possible addition of genetic analysis.

Key words: Myelodysplastic Syndromes; Diagnosis; Classification; Prognosis; Risk Factors; Blood Cells; Bone Marrow Cells

Sažetak

Uvod. Mijelodisplastični sindromi predstavljaju klonske neoplastične bolesti koje se karakterišu krvnom displazijom, neefektivnom hematopoezom, citopenijama i povišenim rizikom transformacije u akutnu mijeloidnu leukemiju. **Materijal i metode.** Pregled literature je obavljen korišćenjem različitih bibliografskih baza podataka: *Google Scholar*, MEDLINE i Kobson. Preporuke za dijagnozu, klasifikaciju i prognozu predstavljaju mišljenje eksperata na osnovu pregleda literature i savremenih preporuka za dijagnozu i prognozu mijelodisplastičnih sindroma. **Dijagnoza i klasifikacija**. Dijagnoza primarnog mijelodisplastičnog sindroma se zasniva na detaljnoj anamnezi uključujući i porodičnu anamnezu, fizikalnom pregledu i sveobuhvatnim laboratorijskim ispitivanjem krvi sa ciljem isključivanja drugih uzroka citopenije i displazija. Obavezni pregledi za postavljanje dijagnoze mijelodisplastičnog sindroma citološki pregledi periferne krvi i koštane srži, biopsija koštane srži sa imunohistologijom i citogenetika. Za postavljanje dijagnoze mijelodisplastičnog sindroma koristi se klasifikacija Svetske zdravstvene organizacije iz 2016. godine. SF1B3 genetska analiza se preporučuje kod bolesnika sa suspektnim mijelodisplastičnim sindromom sa prstenastim sideroblastima, kao i određivanje mutacionog statusa p53 gena. **Prognoza.** Kod svakog bolesnika neophodno je utvrditi skor rizika prema revidiranom međunarodnom prognoznom skoring sistemu za mijelodisplastični sindrom sa ciljem određivanja prognoze bolesti uz moguće dodatne genetske analize. Sekvencionisanje sledeće generacije može da obezbedi dodatne dijagnostičke i prognostičke informacije, posebno kod mlađih bolesnika koji su kandidati za transplantaciju. **Zaključak.** Dijagnoza mijelodisplastičnog sindroma se bazira na klasifikaciji Svetske zdravstvene organizacije iz 2016. godine. Prognoza se zasniva na mijelodisplastični sindrom uz moguće dopunske genetske analize.

Ključne reči: mijelodisplastički sindromi; dijagnoza; klasifikacija; prognoza; faktori rizika; krvne ćelije; ćelije koštane srži

Introduction

Myelodysplastic syndromes (MDS) represent clonal neoplastic disorders characterised by hematological dysplasia, ineffective hematopoiesis with extensive intramedullary apoptosis, cytopenia, and increased risk of transformation to acute myeloid leukemia [1, 2].

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Abbreviations

MDS – Myelodysplastic syndrome/s
IPSS-R – Revised International Prognostic Scoring System
PAS – Periodic acid-Schiff staining
ALIP – abnormal localization of granulocytic precursors
WHO – World Health Organization
FISH – fluorescence in situ hybridisation
NGS – Next-generation sequencing
MDS-RS – a myelodysplastic syndrome with ringed sideroblasts
FCM – Flow cytometry
ELN – European Leukemia Net
ICUS – idiopathic cytopenia of undetermined significance
ARCH – age-related clonal hematopoiesis
CHIP – clonal haematopoiesis of undetermined significance
CCUS – clonal cytopenia of undetermined significance
IPSS – International Prognostic Scoring System
IPSS-M – International Prognostic Scoring System-Molecular

It is of utmost importance to differentiate MDS from other entities and hematological diseases, which may present themselves as different cytopenias; therefore we may say that MDS is the final outcome in the diagnostic work-up of solving this clinical problem.

The incidence of primary MDS is about 3 to 5 cases per 100.000/year [3, 4]. MDS is predominantly a disease of the elderly. The median age is above 70 years [3, 4]. The MDS is more prevalent in males than in females [1–4].

In this paper, we present the recommendations for diagnosis, classification, and prognosis of MDS on behalf of the Serbian MDS group.

Material and Methods

A literature review was conducted using the following bibliographic databases: Google Scholar, MEDLINE. and Kobson. The recommendations for diagnosis, classification, and prognosis are based on expert opinion grounded on a review of the literature and contemporary recommendations for diagnosis and prognosis in MDS.

Diagnosis of myelodysplastic syndrome

As it was said, MDS is the final outcome of diagnostic work-up of patients with different combinations of cytopenia (anemia, leukopenia, thrombocytopenia). In the initial approach, it is very important to evaluate the degree and number of cytopenias. In the case of mild or moderate cytopenia, a thorough clinical evaluation should be performed to provide the answer weather the patient needs further bone marrow evaluation promptly or not. In case of mild blood changes, with mild or unremarcable morphological findings, it is better to arrange frequent follow-ups of the patient than to perform theprompt-ly a detailed hematological evaluation of the bone marrow, producing certain unease in patients and their families. Nutritional deficits should be corrected before any bone marrow diagnostic steps. However, in the case of moderate or severe cytopenia, it is advisable to perform a complete evaluation before any other clinical steps.

Patient history and examination

Patient history should explore symptoms related to cytopenia (anemia, bleeding, and infection), its occurrence in time, if available, as well as information on prior chemotherapy and/or irradiation treatment, occupational exposure to myelotoxic substances such as benzene, nitro. and oil compounds and their derivatives, alcohol-use, concomitant medications [1, 5, 6]. It is also of value to evaluate the possibility of lifelong exposure to different toxic compounds within the household, but also in the environment, like herbicides and pesticides.

If MDS is suspected, detailed family history regarding hematological disorders, cancer, liver and lung diseases, skin diseases, and/or early deaths [1, 5, 6], but also about some other diseases (e.g., germline predispositions) [1, 7] is warranted.

A complete physical examination is needed. The size of the liver and spleen should be determined, enlargement of lymph nodes and some minor somatic abnormalities as well.

After this clinical introduction, a full medical evaluation of the patient includes a comprehensive laboratory examination. It is necessary to exclude other causes of cytopenia and/or dysplasia, mainly nutritional deficits, viral and chronic viral diseases, autoimmune diseases, liver problems with hypersplenism, etc. **(Table 1)** [1, 5, 6, 8].

Examination of peripheral blood and bone marrow

Mandatory examinations in all cases with cytopenia, especially in cases where MDS is suspected, are morphological evaluations of peripheral blood and bone marrow smears [1, 9, 10]. It is essential to assess dysplastic features in all cell lineages, to determine the percentage of blasts in peripheral blood and bone marrow [1], to access iron stores, and the presence of ringed sideroblasts in the bone marrow [1, 11]. The most valuable evaluation, requiring great medical experience, is the determination of blasts type I and II in dysplastic bone marrow [1, 10, 11]. Other compulsory evaluations are bone marrow histology and conventional cytogenetics [1, 5–9].

Dysplasia in a line is determined by finding ≥ 10% of cells with dysplastic features within a certain hematopoietic lineage. We should evaluate all three myeloid lineages (erythroid, granulocytic, and megakaryocytic) and confirm dysplasia in at least one lineage. At least five hundred nucleated bone marrow cells should be counted for blast enumeration and two hundred white blood cells in peripheral blood smears. A hundred erythroblasts should be counted on Pearls (Prussian blue iron staining) in order to determine the presence and quantification of ringed sideroblasts. Besides, at least thirty megakaryocytes should be counted. In case one slide should not be enough, the study should extend to several stained smears [1, 5, 8, 9, 11].

The signs of dyserythropoiesis are numerous, including nuclear budding, internuclear bridging, karyorrhexis, multinuclearity and megaloblastoid changes, poor hemoglobinization (“ghost” erythroblasts), cyto-

Table 1. Required blood tests if myelodysplastic syndrome is suspected
Tabela 1. *Potrebna laboratorijska ispitivanja krvi u slučaju sumnje na mijelodisplastični sindrom*

Haemoglobin, red blood cell indices (MCV, MCH, MCHC, RDW) *Hemoglobin, eritrocitni indeksi (MCV, MCH, MCHC, RDW)*
WBC, manual differential, platelet count, MPV and platelet morphology *Leukociti, ručna diferencijalna formula, MPV i morfologija trombocita*
Relative and absolute reticulocyte count (preferably on automatic analyser) *Relativni i apsolutni broj retikulocita (najbolje sa automatskog brojača)*
Folic acid, vitamin B12, homocysteine (methyl malonic acid if needed). *Koncentracije folne kiseline, vitamina B12, homocisteina (ako je potrebno i metil malonske kiseline)*
standard blood chemistry including urea, creatinine, uric acid, electrolytes (Na, K, Cl, Ca) *standardna biohemija krvi uključujući ureu, kreatinin, mokraćnu kiselinu, elektrolite,* liver tests: bilirubin, ASAT, ALAT, alkaline phosphatase, gGT, proteins, albumin, PT/INR *testove jetre kao ASAT, ALAT, alkalnu fosfatazu, gama GT, proteine, albumin, protrombinsko vreme i INR* Serum iron, transferrin/TIBC, transferrin iron saturation, ferritin, LDH, haptoglobin *Gvožđe u serumu, transferin/TIBC, saturacija transferina, feritin, LDH, haptoglobin* DAT (Coombs test)/*Direktni antiglobulinski, Kobsov test* Serum erythropoietin, Serum protein electrophoresis, IgG, IgM I IgA *Koncentracija eritropoetina u serumu, elektroforeza proteina seruma sa imunoglobulinima G, M i A* *haemoglobin electrophoresis/**elektroforeza hemoglobina*
HIV, hepatitis HBV and HCV screening/*Skrining na HIV, HBV i HCV viruse*
The antinuclear antibody (ANA) immunofluorescence assay (IFA) (“ANA screen”) *Imunofluorescentno testiranje na antinuklearna antitela* Concentration of complements C3, C4, RF, anti CCP/*Koncentracije komplementa C3, C4, reuma faktor i antitela na CCP*
PCR for Parvovirus B19 if hypoplastic MDS is suspected/ *PCR test na parvovirus B19 ako se sumnja na hipoplastični oblik MDS*

plasmic vacuolization, presence of ringed sideroblasts (with basophilic granulation), and Periodic acid-Schiff (PAS) positivity [1, 10, 11]. The hallmark of dysgranulopoiesis is the presence of small or unusually large granulocytes (megaloblastic change), nuclear hyposegmentation (pseudo-Pelger-Huet), rarely nuclear hypersegmentation, decreased granulation of cytoplasm (hypo or agranularity), pseudo-Chediak-Higashi granules, Dohle bodies and the Auer rods (the presence of Auer rods implies an excess of blasts independently of blast numbers) [1, 11]. Megakaryocytic dysplasia is characterized by the presence of micromegakaryocytes, nuclear hypolobation and multinucleation [1, 11].

Bone marrow histology is also a valuable examination, providing additional diagnostic and prognostic information about bone marrow cellularity (e.g., hypocellular variant of MDS) [11, 12], presence of fibrosis, focal infiltration, megakaryocyte morphology, and abnormal localization of granulocytic precursors (ALIPs) [1, 7, 9, 11]. Bone marrow histology can help in excluding focal bone marrow infiltration in case of lymphoma, mastocytosis, metastatic carcinoma, histiocytosis, and other similar diseases [9, 11]. Immunohistohemistry at least for CD34/CD117, is recommended as it can provide additional information/confirmation of blast excess [1, 5–8, 11]. However, the blast enumeration should be based on the cytological examination of peripheral blood and bone marrow according to WHO 2016 Classification [1, 9, 11] and should not be substituted by the percentage of $CD34/CD117^{+}$ cells. The CD34/CD117 immunohistochemistry could be of importance in cases when the bone marrow smear is not representative, e.g., in case of dilution with blood, as well as in case bone marrow aspiration is unsuccessful (try tap) [1, 5, 9, 11]. Other antibody markers within the myeloid panel can also provide additional information about dysplastic features (e.g., abundance of micromegakaryocytes, dislocation of erythroid and granulocytic lineages, etc) [11, 13]. Bone marrow histology is also essential in case of MDS with fibrosis (grade 2 and 3 fibrosis according to WHO) with unfavorable prognosis as well as in cases of hypoplastic MDS, present in up to 10% of patients, who also require immunosuppression treatment [1, 5, 7, 11, 12, 14].

Standard conventional cytogenetics of bone marrow cells should be performed in all patients to confirm the diagnosis (confirm clonality in case of present aberration) and provide classification and prognosis [1, 5, 7–11]. Since myeloid cells in MDS are slow in proliferation due to their biology (and apoptosis in vitro), direct karyotype preparation and short-term culture karyotype are required. Peripheral blood karyotype is used only in case of constitutional abnormalities (e.g., inv (9) or other polymorphisms). At least twenty metaphases should be examined. Some recurrent cytogenetic abnormalities e.g., del(5q), del (7), del(7q), as well as others, with the exclusion of trisomy 8, -Y, and del (20q), are MDS-defining aberrations in a cytopenic patient, even without morphological dysplasia [1, 7, 11, 15], therefore leading to the term “genetically defined MDS”.

Conventional cytogenetics is not always successful. In these cases, fluorescence in situ hybridisation (FISH) analysis of marrow or, rarely, peripheral blood is recommended for typical MDS defining aberrations (e.g. del 7, del(5q), trisomy chromosome 8) [5, 9, 15].

SF3B1 mutation analysis is recommended in the cases of MDS with the presence of ringed sideroblasts [1, 5–9]. It is mandatory in the cases with ≥5% and <15% ringed sideroblasts to confirm the diagnosis of myelodysplastic syndrome with ringed sideroblasts (MDS-RS) according to the WHO classification but not in cases with >15% of ring sideroblasts [1, 5–9].

Next-generation sequencing (NGS) is recommended in transplant candidates [8, 15], but it is believed that it might provide a better prognostic understanding of all patients in the future.

Flow cytometry (FCM), mainly of bone marrow cells, is not a mandatory evaluation, but in a certain number of cases, it can contribute to the diagnosis and the prognosis of MDS patients [6, 7, 11, 16, 17]. Guidelines recommend that FCM should be done only in centers with high expertise in FCM in MDS and AL according to ELN recommendations [4, 8, 9]. Aberrant expression of different antigens and their disturbed maturation pattern on flow cytometry could help to distinguish MDS in challenging cases [16]. FCM could help in cases of hypoplastic MDS, but also to evaluate the presence of PNH clones, which is not rare in MDS patients. Moreover, FCM can also help in the evaluation of immunological abnormalities in a spectrum of autoimmunity within MDS entities [6, 14, 16].

In some patients with immunological changes, but also with cytopenia and some morphological features of MDS, the use of clonogenic *in vitro* assay can help in distinguishing the type of growth of bone marrow cells and colonies and determining some possible immunological influence on hematopoiesis [18].

After a comprehensive evaluation of the suspect MDS patients, the diagnostic procedure is ended by establishing a diagnosis of primary MDS according to current classification criteria by the World Health Organization (WHO 2016) **(Table 2)** [1, 11]. New approaches toward the classification of MDS are underway by two independent expert groups, International Consensus Classification [7] and 5th WHO Appointed Editorial Panel [15], but the main goal of both is to incorporate more genetics into basic classification and, in many cases, to allow easier access to treatment by modern, but sometimes aggressive, therapies including stem cell grafting.

In the evaluation of MDS patients, hereditary disorders should be considered in younger patients (< 50 years), but also in patients with multiple cancers, or positive family history (first and second degree relatives), preceding prolonged thrombocytopenia, as well as with associated abnormalities like nail dystrophy, short stature, presence of lymphedema, liver, pancreas and pulmonary conditions which are not easily explained [1, 4–9], especially to be able to exclude genetic, germline predisposition in search for a potential transplant donor.

These conditions include congenital dyserythropoietic anemia, telomere-associated syndromes including congenital dyskeratosis, hereditary sideroblastic anemia, Fanconi anemia, congenital neutropenias (Kostmann, Schwachman-Diamond syndromes), Diamond-Blackfan anemia, familial platelet disorders including those with RUNX1 and GATA2-Mutations. All these conditions are described as Myeloid neoplasms with germline predisposition according to both the current 2016 WHO classification and new proposals [1, 7, 15].

Clonal cytopenia of undetermined significance and associated conditions

Investigation of the biology of MDS, especially in elderly patients, revealed that a certain number of them develop specific clonal changes in hematopoiesis, especially those detected on the molecular level, but also that there are patients with cytopenia without other signs of frank MDS. One of these conditions is named idiopathic cytopenia of undetermined significance (ICUS) and could be diagnosed in patients with at least one cytopenia without morphology of MDS defined dysplasia, no excess of blasts, absence of MDS-related genetic aberrations or evidence of other hematological disease [19]. The ICUS patients should be followed because of the near 10% risk of developing myeloid malignancies after 10 years.

Another condition is called age-related clonal hematopoiesis (ARCH) and it is diagnosed when somatic mutations associated with myeloid malignancies are found in blood or bone marrow without signs of hematological disease [20]. The ARCH becomes frequent with older age and could be found in more than 10% of the population over 70 years [20]. However, the risk of myeloid malignancy is low, about 1% per year. The other possible term for this condition is clonal hematopoiesis of undetermined significance (CHIP) [19, 20].

The diagnosis of clonal cytopenia of undetermined significance (CCUS) could be established in patients with at least one cytopenia and clonal hematopoiesis without criteria for MDS or other haematological diseases. The risk of developing myeloid malignancy, including MDS, is exceptionally high [19, 21].

Sometimes, morphology features are present together with cytopenia, but even thorough genetic evaluation, including NGS myeloid panel sequencing, was not able to provide any kind of clonal hematopoietic lesion [22]. We should be very cautious in all aspects of the approach, first of all, in order to avoid any deterioration of hematopoiesis, especially significantly a rise in the blast count leading towards acute leukemia and aggressive therapy, and second, to prepare the field for possible stem cell grafting in case any genetic event is uncovered during follow up.

Prognosis of MDS

The current MDS classification (WHO 2016) [1] is not intended to replace its terms and hierarchy and prognostic scoring systems developed over time. Even new classification proposals do not have such intention, even if they incorporate some prognostic variables in their structure and definition. Currently, The Revised International Prognostic Scoring System (IPSS-R) as a dynamic score system proven to as superior in comparison to the previous International Prognostic Scoring System (IPSS); therefore, it is rec-

Table 2. Current World Health Organization 2016 classification of Myelodysplastic syndrome
Tabela 2. *Trenutna klasifikacija mijelodisplastičnog sindroma prema 2016 klasifikaciji Svetske zdravstvene organizacije*

Entity *Naziv grupe*	Number of dysplastic lineages/*Broj displastičnih krvnih loza*	Number of cytopenia*/*Broj citopenija**	Ring sideroblasts as percentage of marrow erythroid elements *Prstenasti sideroblasti kao procenat eritroblasta*	Bone marrow and peripheral blood blasts/*Blasti u koštnoj srži (KS) i perifernoj krvi (PK)*	Cytogenetics *Citogenetika*
MDS with single lineage dysplasia (MDS-SLD)/*MDS sa displazijom jedne krvne loze (MDS-SLD)*	1	1-2	<15%/<5%**	BM/KS < 5%, PB/PK < 1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)/*Bilo koji, osim ako nisu ispunjeni svi kriterijumi za MDS sa izolovanom del(5q)*
MDS with multilineage dysplasia (MDS-MLD)/*MDS sa multilinijskom displazijom (MDS-MLD)*	2 -3	1-3	<15%/<5%**	BM/KS < 5%, PB/PK < 1%, no Auer rods	
MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD)/*MDS sa prstenastim (ring) sideroblastima i diplazijom jedne krvne loze (MDS-RS-SLD)*	1	1-2	≥15%/≥5%**	BM/KS < 5%, PB/PK < 1%, no Auer rods	
MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD)/*MDS sa prstenastim (ring) sideroblastima multilinijskom displazijom (MDS-RS-MLD)*	2-3	1-3	≥15%/≥5%**	BM/KS < 5%, PB/PK < 1%, no Auer rods	
MDS with isolated del(5q) *MDS sa izolovanom el(5q)*	1-3	1-2	none or any bez ili bilo koji	BM/KS < 5%, PB/PK < 1%, no Auer rods	del(5q) alone or with one additional abnormality, except -7 or del(7q) del(5q) kao jedina promena ili sa jednom dodatnom promenom sem -7 ili del(7q)
MDS with excess blasts (MDS-EB)/*MDS sa viškom blasta (MDS-EB)*					
MDS-EB-1	0-3	1-3	none or any *bez ili bilo koji*	BM/KS 5-9% or/ili PB/PK 2-4%, no Auer rods	any *bilo koja*
MDS-EB-2	0-3	1-3	none or any *bez ili bilo koji*	BM/KS 10-19% or/ili PB/PK 5-19% or/ili Auer rods present/prisutni	any *bilo koja*
MDS, unclassified (MDS-U)/*MDS, neklasifikovan (MDS-U)*					
With 1% of blasts in PB *Sa 1% blasta u PK*	1-3	1-3	none or any *bez ili bilo koji*	BM/KS < 5%, PB/PK = 1%,‡ no Auer rods	any *bilo koja*
With uni-lineage dysplasia and pancytopenia *Sa unilinijskom displazijom i pancitopenijom*	1	3	none or any *bez ili bilo koji*	BM/KS < 5%, PB/PK < 1% no Auer rods	any *bilo koja*
MDS-U based on a cytogenetic abnormality typical for MDS/*MDS-U zasnovan na citogenetskom poremećaju tipičnom za MDS*	0	1-3	< 15%§	BM/KS < 5%, PB/PK < 1% no Auer rods	MDS-defining abnormality *Citogenetski poremećaj tipičan za MDS*
Provisional entity: Refractory cytopenia of childhood/*Refrakterna citopenija u detinjstvu*	1-3	1-3	none *bez*	BM/KS < 5%, PB/PK < 2%	any *bilo koja*

*cytopenia is defined as haemoglobin < 100 g/L, Platelet count < $100x10^9$/L, absolute neutrophil count, ANC < $1.8x10^9$/L, although MDS can be present with anaemia and/or thrombocytopenia above those levels. Absolute monocyte count < $1x10^9$/L
**citopenija se definiše kao hemoglobin < 100 g/L, broj trombocita < $100x10^9$/L, apsolutni broj neutrofila, ABG < $1.8x10^9$/L, mada MDS može biti prisutan i sa anemijom i/ili trombocitopenijom iznad tih granica. Apsolutni broj monocita < $1x10^9$/L*
** if SF3B1 mutation present/*ako je prisutna SF3B1 mutacija*
‡ blast in PB recorded in at least two occasions/*blasti prisutni u perifernoj krvi u najmanje 2 merenja*

Table 3. Revised International prognostic scoring system (R-IPSS)*
Tabela 3. *Revidirani Internacionalni prognostički bodovni sistem (R-IPSS)**

Prognostic factors/*Prognostički parametri*	0	0.5	1	1.5	2	3	4
Cytogenetics#/*Citogenetika#*	very good *veoma dobra*	–	good *dobra*	–	intermediate *intermedijerni*	poor *loša*	very poor *veoma loša*
Blasts in bone marrow (%) *Blasti u kostnoj srži (%)*	≤ 2	–	> 2 - < 5%	–	5 - 10%	> 10%	–
Haemoglobin (g/L)/*Hemoglobin (g/L)*	≥ 100	–	80 - < 100	< 80	–	–	–
Platelets (x10^9/L)/*Trombociti (x10^9/L)*	≥ 100	50 - < 100	< 50	–	–	–	–
ANC (x100^9/L)/*ABG (x10^9/L)*	≥ 0.8	< 0.8	–	–	–	–	–

* **interpretation of risk score**: very low risk ≤ 1.5; low risk > 1.5-3; intermediate risk > 3-4.5; high risk > 4.5-6; very high risk > 6
* ***tumačenje bodovnog sistema****: veoma nizak rizik ≤ 1.5; nizak rizik >1.5 – 3; intermedijerni rizik >3-4.5: visok rizik > 4.5-6, veoma visok rizik > 6*
# cytogenetics risk groups: very good: -Y, del(11q); good: normal karyotype, del(5q), del(12p), del(20q), double including include del(5q); intermediate: del(7q), +8, +19, i(17q), any other single or double independent clones; poor: -7, inv(3)/t(3q), double including -7/del(7q), complex: three abnormalities; very poor: complex karyotype: >3 abnormalities

ommended in estimating the the risk of progression and death in particular patients **(Table 3)** [23–26]. The such risk scores can also be used from different internet sites, including the one of the MDS Foundation, enabling physicians to provide an estimate of the disease of a patient at any time their. Besides, it is of value to calculate previous IPSS scores, since many drugs currently in use are designated and approved according to trials using IPSS in the past (Eg. azacytidine, lenalidomide, luspatercept, erythropoietin).

Both scoring systems include exact blast count from the bone marrow and cytogenetic aberrations found, and thus in some instances they can replace the current classification. We are taking about several degrees of risk: low risk, intermediate 1 and 2, and high risk, with the main risk impact of the blast count and very complex karyotype.

Low-risk MDS includes patients with low and intermediate-1 IPSS, but also very low IPSS-R, low, and intermediate up to 3.5 points. High-risk MDS includes those with intermediate-2 and high risk IPSS, as well as intermediate > 3.5 IPSS-R, high, and very high [27–30]. The IPSS-R should be used to evaluate prognosis in all patients [5].

The introduction of different molecular evaluations in MDS patients, especially NGS myeloid panel sequencing, led to the introduction of a specific prognostic model named International Prognostic Scoring System-Molecular (IPSS-M), based on previous IPSS-R model and incorporating the results of genetic analysis [21]. A six-category risk model includes very low (14%), low (33%), moderately low (11%), moderately high (11%), high (14%), and very high risk groups (17%) of patients. The application of IPSS-M has shown significant prognostic improvement when compared with IPSS-R [21] but it deserves further validation.

Conclusion

Myelodysplastic syndromes diagnosis is a part of diagnostic work-up in patients with cytopenia. Diagnosis is based on a comprehensive evaluation, including history and physical examinations, along with blood and bone marrow investigation. The myelodysplastic syndromes diagnosis should be confirmed by the presence of cytopenia, dysplastic features, and proportion of blast cells according to morphology analysis of peripheral blood and bone marrow. Bone marrow histology and cytogenetics are also mandatory because they can provide additional diagnostic and prognostic information.

The SF3B1 mutation, but also p53 mutation evaluations are recommended for cases of acquired sideroblastic anemia or high-grade myelodysplastic syndromes, respectively. Next-generation sequencing is recommended for patients who are transplant candidates. Revised International Prognostic Scoring System should be used to evaluate prognosis in all patients.

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TREATMENT OF LOWER-RISK MYELODYSPLASTIC SYNDROME

LEČENJE MIJELODISPLASTIČNOG SINDROMA NISKOG RIZIKA

Dragomir MARISAVLJEVIĆ[1], Aleksandar SAVIĆ[2, 3] and Andrija BOGDANOVIĆ[4, 5]

Summary

Introduction. We present the recommendations for treatment of the lower-risk myelodysplastic syndromes on behalf of the Serbian myelodysplastic syndromes group. **Material and Methods.** A literature review was conducted using the following bibliographic databases: Google Scholar, MEDLINE and Kobson. The recommendations for treatment of lower-risk myelodysplastic syndromes are based on expert opinion based on review of the literature and contemporary recommendations for treatment of lower risk myelodysplastic syndromes. **Recommendations.** Anemia is the most relevant cytopenia in terms of frequency and symptoms in lower-risk myelodysplastic syndromes, and may be treated successfully with erythropoietic stimulating agents, with or without granulocyte growth factor, provided a careful selection is performed on the basis of Revised International Prognostic Scoring System, endogenous erythropoietin levels, and transfusion independence. In case a patient fails erythropoietic stimulating agents treatment, the available options may include lenalidomide, hypomethylating agents, and a rather large number of experimental agents. Chelation therapy is recommended in patients who have received or are anticipated to receive > 20 red blood cell transfusions and those with serum ferritin levels > 2500 ng/mL. Specific therapy for thrombocytopenia has been proposed in experimental clinical trials with thrombomimetic agents that have shown good efficacy, but raised some safety concern. Severe neutropenia is targeted symptomatically with growth factor supportive care. The immunosuppressive treatments are indicated mainly for pancytopenia, hypoplastic lower-risk myelodysplastic syndromes. Finally, hematopoietic stem cell transplantation is the curative option for younger, good performance (fit) lower-risk patient with poor risk features, according to European Blood and Marrow Transplantation/European Leukemia Net International expert panel and myelodysplastic syndrome-RIGHT group. **Conclusion.** Treatment of myelodysplastic syndromes is mainly based on resolution of symptoms due to particular cytopenia(s).

Key words: Myelodysplastic Syndromes; Therapeutics; Risk Assessment; Risk Factors; Treatment Outcome

Sažetak

Uvod. U radu su predstavljene preporuke za lečenje mijelodisplastičnih sindroma niskog rizika u ime srpske MDS grupe. **Materijal i metode.** Pregled literature je obavljen korišćenjem sledećih bibliografskih baza podataka: *Google Scholar*, *MEDLINE* i *Kobson*. Preporuke za lečenje mijelodisplastičnih sindroma niskog rizika zasnovane su na osnovu pregleda literature i savremenih preporuka za lečenje mijelodisplastičnih sindroma niskog rizika. **Preporuke.** Anemija je najrelevantnija citopenija u smislu učestalosti i simptoma kod mijelodisplastičnih sindroma niskog rizika, i može se uspešno lečiti agensima koji stimulišu eritropoezu (*Erythropoiesis-Stimulating Agent*), sa granulocitnim faktorom rasta ili bez, pod uslovom da se izvrši pažljiva selekcija bolesnika na osnovu Revidiranog međunarodnog sistema prognostičkog bodovanja, nivoa endogenog eritropoetina i zavisnosti od transfuzije. U slučaju neuspeha lečenja eritropoeze, raspoložive opcije su primena lenalidomida, hipometilirajućih agenasa i značajnog broja eksperimentalnih agenasa. Terapija helatorima gvožđa preporučuje se kod bolesnika koji su primili ili se očekuje da će primiti > 20 transfuzija koncentrovanih eritrocita i pacijenti sa nivoima feritina u serumu > 2500 ng/mL. Trombomimetički agensi pokazali su značajnu efikasnost u lečenju teške trombocitopenije u kliničkim studijama, ali uz prisustvo određenih bezbednosnih rizika. Teška neutropenija sa ponavljanim ozbiljnim infekcijama leči se simptomatski uz pomoć faktora rasta. Imunosupresivno lečenje indikovano je samo za bolesnike sa pancitopenijom i hipoplastičnim mijelodisplastičnih sindroma. Konačno, alogena transplantacija matičnih ćelija hematopoeze je kurabilna terapijska opcija za mlađe bolesnike sa dobrim performansom, ali sa karakteristikama nepovoljnog rizika prema međunarodnom ekspertskom panelu Evropska grupa za transplantaciju krvi i kostne srži/Evropska mreža za leukemiju i MDS-RIGHT grupi. **Zaključak.** Lečenje mijelodisplastičnih sindroma niskog rizika uglavnom je zasnivano na otklanjanju ili ublažavanju simptoma do kojih dovode određene citopenije.

Ključne reči: mijelodisplastički sindromi; terapija; procena rizika; faktori rizika; ishod lečenja

Introduction

For more than a quarter of a century, according to the International Prognostic Scoring System (IPSS) and Revised-IPSS, hematologists have divided myelodysplastic (MDS) patients into two basic risk categories: "low-risk" (IPSS score ≤ 1 or IPSS-R score ≤ 3.5) and "high-risk" patients. Following the risk assessment, appropriate therapeutic options are applied, taking into account the individual characteristics of the patient, such as the presence of comorbidities, age and suitability for

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hematopoietic stem cell transplantation (HSCT) [1–3]. In addition, it is of extreme importance to identify those lower-risk MDS patients who would benefit from an earlier treatment, particularly in the light of recent efforts towards integration of clinical variables with certain biological characteristics, such as somatic mutations [1, 3]. Lower-risk patients harboring mutations of TP53, EZH2, ASXL1, CBL, and U2AF1 will fare worse than predicted by disease risk. On the contrary, SF3B1 mutations that are frequent in ring sideroblasts (RARS) and RARS associated with marked thrombocytosis, and myelodysplastic/myeloproliferative neoplasms subtypes are associated with longer overall survival (OS) than that calculated by the IPSS-R [1, 3]. It is clear that although sharing the same IPSS-R score, the patients carrying different mutations would require a different treatment approach [1]. Recently, the European Hematological Association (EHA) has strongly endorsed the MDS-RIGHT group guidelines on myelodysplastic syndromes (MDS) [4]. The MDS-RIGHT project is a large European project funded under the Horizon 2020 program which relies on the European MDS Patient Registry and aims to provide the proper care to the right MDS patient at the right time and develop evidence-based guidelines [4], mainly from ELN [5].

It is important to stress that the majority of patients with MDS have an IPSS lower-risk disease: in the Italian National MDS registry (FISMonlus) [6] among a total of 4300 MDS cases, 20% have IPSS-R very low, 41% low, and 19% intermediate. Since treatment of MDS is mainly based on the resolution of symptoms due to cytopenias, for simplicity, treatment of single cytopenias will be particularly addressed.

Material and Methods

A literature review was conducted using the following bibliographic databases: Google Scholar, MEDLINE and Kobson. The recommendations for are based on expert opinion based on a review of literature and contemporary recommendations for treatment of lower-risk MDS.

Supportive Care

Currently, the standard of care for MDS management includes supportive care measures [1]. This entails observation, clinical monitoring, psychosocial support, and quality of life (QoL) assessment. Significant efforts should be directed toward addressing the relevant QoL domains (ex, physical, functional, emotional, spiritual, social), which negatively affect the patient [1]. Supportive care should include RBC transfusions for symptomatic anemia as needed (CMV-safe) or platelet transfusions for bleeding events. Both the number of transfusions as well as the number of packed RBCs per transfusion should be kept to a minimum in non-cardiac patients and in patients anticipated to be heavily transfused [1]. In addition, there are differences in the approach to transfusion therapy between countries. In many countries, transfusions are not performed to obtain normalization of the Hb level, and require a lower threshold of 8 g/dL or less (unless significantly symptomatic).

Treatment of anemia

Anemia is the most common cytopenia in MDS, present in 89% of the cases and is the major determinant of symptoms in low and Int-1 IPSS, and low and very low IPSS-R MDS [6]. With a median age of 71 to 74 years for MDS patients in Western countries (Italian MDS National Registry (FISMonlus) Available at: www.fismonlus.It) it is evident that chronically low levels of hemoglobin (Hb) can severely impact already frail subjects, causing not only the worsening of cardiac function, but also increasing falls and inducing cognitive impairment [6]. Therefore, anemia of MDS represents an important social and economic burden [1].

The first choice of treatment should be erythropoiesis-stimulating agents (ESAs), such as recombinant human Epo (rHu Epo) or the longer-acting darbepoetin, with or without G-CSF. Erythroid response rates of 40% to 60% (combined major and minor responses using IWG response criteria) have been seen in clinical studies, particularly in patients with lower-risk MDS [1]. In practice, anemic lower-risk MDS patients should start treatment with fixed doses, rather than weight-adjusted doses of ESAs. The optimal treatment doses are 30-80.000 U of EPO [7] and 150-300 mg of darbepoetin subcutaneous (s.c.) injection per week [8]. Probability of respond is higher for early treated, transfusion- independent patients [9]. Response to ESAs is generally observed within 12 weeks and should not be evaluated before then to avoid missing some cases that show later increases of Hb [10]., ESA doses can be tapered when a positive response is achieved to reach the lowest effective dose to maintain Hb. The highest rates of response (as much as 70%) can be obtained if particular selection criteria are followed [10]: endogenous EPO levels should be < 500 U/L according to the Nordic score [11] or even better if EPO levels are < 200 U/L, transfusion requirement should be absent or limited (< 2 U/month), cytogenetics normal and marrow blasts absent [10, 12] **(Table 1)**. Other determinant of response is low serum ferritin level [1], whilst recurrent individual somatic mutations (SF3B1, TET2, ASXL1, and DNMT3A) had no impact on response to ESAs, nor had the size of the mutated clones [13].

During treatment with ESAs, MDS lower-risk patients did not experience an increase of thrombotic events compared with non-treated patients [14], which is quite different to what was observed for other hematologic neoplasias. It is, therefore, not necessary to establish any antithrombotic therapy, unless in the presence of thrombophilia. In addition, Jadersten et al. reported improved survival in low-risk MDS patients with low transfusion need following EPO+G-CSF treatment [15]. Another study found that IPSS low or int-1 patients who received Epo

medication along with or without G-CSF fared better than the historical control IMRAW database patients in terms of survival and AML progression [16]. Given these data, the NCCN Panel recommends the use of ESAs in the management of symptomatic anemia in MDS patients, with a target hemoglobin range of 100-120 g/L but not exceeding 120 g/L.

Response duration to ESAs ranges from 20 to 24 months [16] and response is present but shorter for lower-risk MDS cases with del(5q) [1, 3]. Increased responsiveness, notably in RARS, may be attributed to the addition of G-CSF to ESAs [17].

At failure/relapse to ESAs, bone marrow aspiration must be performed to exclude disease progression, as well as evaluation of iron balance, and of vitamin B12 and folate levels [1, 3].

MDS lower risk with del(5q)

Lenalidomide is FDA-approved for the treatment of MDS patients with transfusion-dependent anemia caused by low-/Int-1-risk MDS del(5q), whether or not there are additional cytogenetic abnormalities. However, in Europe, its use is limited to IPSS low-/Int-1 MDS with an isolated del(5q), when other therapeutic options (such as EPO and G-CSF) are insufficient or ineffective [1, 3]. Lenalidomide is used at the starting dose of 10 mg per day for 21 days on a 28-day cycle. The 10-mg dose is more effective than the 5-mg dose in inducing transfusion independence (61% vs. 49%), and cytogenetic response, with a similar safety profile [1–3]. Achievement of durable transfusion independence with lenalidomide was associated with a significantly reduced risk of AML progression and death [18, 19]. Lenalidomide treatment is maintained until loss of response [1].

Very few lower-risk MDS del(5q) patients, i.e., those with severe renal insufficiency and those with significant cytopenias in addition to anemia, are ineligible for lenalidomide treatment. Half of the patients start responding to lenalidomide after the first cycle [18]. Karyotype complexity has a negative impact both on OS and AML evolution of lenalidomide treated patients [19]. A negative impact on OS is also due to TP53 mutations, which are more frequent in isolated del5q (19%) and complex karyotype with -5/5q- (72%) [20]. Therefore, the assessment of TP53 mutations should be implemented in clinical practice to plan subsequent treatment strategies.

MDS Lower Risk Without Del(5Q)

Patients with lower-risk MDS without del(5q) who fail or relapse after ESAs have limited therapeutic options, frequently need ongoing transfusions, and have a relatively poor quality of life [1]. In fact, hypomethylating drugs (HMTs) are approved in the United States in this setting, but there are no approved treatments in the European Union. Thus, a substantial number of lower-risk MDS patients could be candidates for supportive therapy or experimental drugs [1–3].

HMTs, azacitidine, and decitabine have shown efficacy in lower-risk MDS patients both as first-line and second-line therapy [21, 22]. Both drugs are active in inducing transfusion independence and hematologic improvement in up to 60% of treated patients. There are no final data on their impact on OS in lower-risk MDS, nor on the duration of response and treatment. Published studies have employed standard as well as "adapted" (i.e. lower) doses and schedules of HMTs with apparently similar results in terms of hematologic improvement [21–23].

Lenalidomide has been used in non-del5q lower-risk MDS patients ineligible for or refractory to ESAs and its efficacy at 10 mg per day vs. placebo was recently evaluated in transfusion-dependent lower risk MDS patients [24]. Transfusion independence was obtained in 26.9% of the cases, but a subgroup of patients with endogenous EPO levels < 100 U/L who had received previous ESA treatment reached transfusion independence in 42.5% of the cases [24]. No somatic mutation predicts for response [24].

Transforming growth factor-beta activation in MDS contributes to impairment of erythropoiesis: the promising strategy of blocking this pathway was evaluated more recently with luspatercept (the modified activin receptor IIB containing molecule ACE-536). Luspatercept inhibits SMAD2/3 signaling by blocking GDF11 when administered in ESA-resistant lower-risk MDS patients, induced transfusion independence in 40% of cases and hematologic improvement in 70%, the majority response being the RARS subtype of MDS [25]. The efficacy and safety of luspatercept were evaluated in a Phase 3 multicentre, randomised, double-blind, placebo-controlled study MEDALIST (ACE-536-MDS-001) in adult patients with transfusion-dependent anemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts, who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy. According to obtained results, luspatercept was approved in June 2020 by the EMA for TD LR-MDS-RS patients who are refractory or resistant to ESAs under the trademark Reblozyl™ (25 mg and 75 mg powder for solution for injection). Luspatercept is given as a sc injection og 1.0 mg/kg every 3rd week. The dose can be increased to 1.33 mg/kg if the patient is not transfusion free after 2 consecutive doses, and it can be increased to 1.75 mg/kg if the patient still requires transfusions after 2 consecutive injections.

Treatment of neutropenia

Isolated neutropenia as the only manifestation of the disease is rare. It can be among the different subtypes of MDS according to the WHO classification, and such cases have a good prognosis and a low rate of transformation into AML [26]. On the other hand, the functional defects of dysplastic neutrophils, together with the burden of the organism with iron and the inefficiency of B and T lymphocytes and NK cells, may together contribute to the unexpectedly high susceptibility rate to infection in MDS. Although G-CSF is widely used in the treatment of febrile neutropenia and sporadically in the treatment of severe neutropenia, or in combina-

tion with ESAs and during treatment with hypomethylating agents, there is no evidence that its use affects the natural course of MDS, in terms of overall survival, progression to AML or even in term of the prevention of infections [27]. Overall, most MDS patients who succumb to infections are in the high-risk category [1–3].

Treatment of thrombocytopenia

Severe thrombocytopenia and subsequent fatal bleeding are the immediate cause of death in 13% of patients with low-risk MDS [28]. The frequency and severity of bleeding in patients are increased by functional/qualitative defects of dysplastic platelets, as well as concomitant medication common to the elderly (acetylsalicylic acid, antirheumatic drugs). The severity of thrombocytopenia has been proven to have independent prognostic significance in MDS [29, 30]. Unfortunately, platelet transfusions are highly immunogenic and have a short-term beneficial clinical effect and contribute to the development of splenomegaly. Also, some treatment modalities (use of hypomethylating agents or lenalidomide) may initially worsen thrombocytopenia and consequently increase the risk of fatal bleeding.

A number of drugs that actively promote megakaryocytopoiesis have been investigated in MDS to date, but only two have shown clinical efficacy. Romiplostim is a stimulatory thrombopoiesis Fc-fusion peptide protein administered as s.c. injections. Romiplostim stimulates platelet formation via the TPO receptor without competing with TPO and is approved for the treatment of immune thrombocytopenic purpura (ITP). Romiplostim was tested in a randomized phase 2 study of 250 LR-MDS patients treated with s.c. dosing vs. placebo. Platelet RRs were 36.5% vs. 3.6%, respectively, with the incidence of bleeding events and platelet transfusions significantly reduced in the romiplostim group vs. placebo [31]. Although the trial was stopped because of concerns related to excess blasts and progression to AML in the romiplostim arm, 5-year follow-up data did not demonstrate an increased risk of AML or death [32].

Eltrombopag, a synthetic non-peptide TPO-receptor agonist that can be taken orally, varies significantly from romiplostim in terms of both its structure and mode of action. It binds to both the TPO receptor's transmembrane and juxtamembrane domains. It is approved for the treatment of ITP and aplastic anemia. Eltrombopag efficacy was evaluated in severely thrombocytopenic (platelets < 30 x 10^9/L) IPSS lower-risk MDS patients in prospective, placebo-controlled, single-blind study. Eltrombopag - treated patients had significantly lower bleeding events (14% vs. 42%), and higher platelet rate response (47% vs. 3%) compared with placebo. Median time to response was two weeks [33].

Both romiplostim and eltrombopag have been used in combination with HMTs to decrease severity of thrombocytopenia seen during the initial cycles [1]. Romiplostim was demonstrated as active in reducing platelets nadir and decreasing bleeding events during azacitidine and decitabine treatments [34, 35]. The association of romiplostim and lenalidomide used in the attempt to decrease the frequency of dose reductions/delays due to thrombocytopenia, induced a trend toward lower numbers of transfusions in romiplostim arm [36]. In summary, TPO is active in stimulating very early hematopoietic progenitor cells. It is, therefore, not surprising that active thrombomimetic drugs do increase the number of blasts in the bone marrow. Until we ascertain that the effect of thrombomimetic agents on blasts is transient, in analogy to what was observed for G-CSF, they should be used with caution [1–3].

Treatment of pancytopenia

Patients with 2 to 3 cytopenias remain categorized as lower risk only if they have normal cytogenetics and no blasts in the marrow. Azacitidine and decitabine are an option in these cases, but antithymocyte globulin (ATG)-responsive MDS can be quite well identified as those with hypocellular marrow, without increased blasts, normal karyotype, HLA-DR 15-positivity, and STAT-3 mutant cytotoxic T-cells [1]. IPSS scoring has no impact on hematologic improvement [37]. An analysis of 129 patients treated with equine ATG alone, cyclosporine alone, or in combination showed markedly improved response rates in the subgroup of patients 60 years of age or younger with IPSS int-1 risk or patients with high response probability characteristics as indicated by their prior criteria (ie, age, number of transfusions, possibly HLA-DR15 status) [38]. However, unlike aplastic anemia, results from clinical studies do not always demonstrate that MDS treated with immunosuppression have OS advantage [37].

Management of Iron Overload

RBC transfusions are a vital component in the supportive care of MDS patients. Although the specific therapies patients receive may alleviate RBC transfusion need, a substantial proportion of MDS patients may not respond to these treatments and may develop iron overload and its adverse effects particularly, on hepatic, cardiac, and endocrine function [39]. In a meta-analysis including eight observational studies patients receiving iron chelation therapy had a longer median survival time compared to patients who did not receive therapy [40]. Thus, effective treatment of transfusional siderosis in MDS patients may be necessary.

For patients with chronic RBC transfusion need, serum ferritin levels and associated organ dysfunction (heart, liver, and pancreas) should be monitored, preferably using SQUID (Superconducting Quantum Interference Device), or more recently T2* MRI [1].

Currently available iron chelators as deferoxamine (given as intramuscular or subcutaneous injections) and deferasirox (given orally) [1]. A large, multicenter, phase III, randomized controlled trial is currently underway to evaluate outcomes of deferasirox compared with placebo in patients with MDS; the primary endpoint of this ongoing study is event-free survival (reg-

istered at clinicaltrials.gov; NCT00940602). In addition, there are ongoing clinical trials in patients with MDS receiving oral iron chelating agents to address whether iron chelation alters the natural history of transfusion dependent patients.

The NCCN Guidelines Panel recommends consideration of once-daily deferoxamine subcutaneously or deferasirox orally to decrease iron overload (aiming for a target ferritin level less than 1000 ng/mL) in the following IPSS low- or int-1–risk patients: 1) patients who have received or are anticipated to receive greater than 20 RBC transfusions; 2) patients for whom ongoing RBC transfusions are anticipated; and 3) patients with serum ferritin levels greater than 2500 ng/mL [1].

Following post-marketing use of deferasirox, there were case reports of acute renal failure, hepatic failure or cytopenias (including agranulocytosis, neutropenia, and thrombocytopenia, and GI bleeding), some of which were fatal. There is currently no evidence linking such episodes to deferasirox therapy [1]. However, it is recommended that patients on deferasirox therapy be closely monitored. Monitoring should include measurement of serum creatinine and/or creatinine clearance and liver function tests prior to initiation of therapy and regularly thereafter. Deferasirox and deferoxamine should be avoided in patients with creatinine clearance less than 40 mL/min.

Recommendations

1. Consider allogeneic stem cell transplantation in the case of younger, good performance (fit) lower-risk patient with poor risk features according to EBMT/ELN International expert panel and MDS right group (poor-risk genetic features, bone marrow fibrosis, intense transfusion need, severe thrombocytopenia or neutropenia).
2. For patients with symptomatic anemia, consider EPO ± G-CSFfor patients with predictive score 0 or 1 according to the predictive model with a target hemoglobin range od 100-120 g/L.
3. High-quality transfusion and chelation therapy in patients who have received or are anticipated to receive > 20 RBC transfusions; and patients with serum ferritin levels > 2500 ng/mL.
4. Evaluate patients with MDS with single lineage dysplasia and MDS with multiple lineage dysplasia for immunosuppressive treatment.
5. Lenalidomide treatment for patients with IPSS-R low and intermediate risk MDS with isolated del(5q), who have failed growth factor treatment or are not eligible for this treatment according to the predictive model, and who are not p53 positive by immunohistochemistry.
6. Patients with severe cytopenia and/or transfusion dependency who have failed other relevant therapies should be considered for experimental treatment within a clinical trial [2].

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TREATMENT OF HIGHER-RISK MYELODYSPLASTIC SYNDROME

LEČENJE MIJELODISPLASTIČNOG SINDROMA VISOKOG RIZIKA

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Summary

Introduction. The myelodysplastic syndromes are a group of clonal haematopoietic stem cell disorders characterized by cytopenia, dysplasia, ineffective hematopiesis, recurrent genetic abnormalities, and increased risk of developing acute myeloid leukemia. In this paper, we present the review and recommendations for treatment of high risk myelodysplastic syndromes on behalf of the Serbian myelodysplastic syndromes group. **Material and Methods.** A literature review was conducted using the following bibliographic databases: Google Scholar, MEDLINE and Kobson. The recommendations treatment of high risk myelodysplastic syndromes are based on expert opinion based on review of literature and contemporary recommendations for treatment of high risk for myelodysplastic syndromes. **Recommendations.** Higher-risk myelodysplastic syndromes should be defined in patients risk group with > 3.5 IPSS-R score. Allo-HSCT is recommended in fit higher-risk patients with IPSS-R > 3.5 as well as in fit lower-risk patients with poor risk features according to EBMT/ELN International expert panel and myelodysplastic syndromes right group. Acute myeloid leukemia like or hypomethylation treatment before Allo-HSCT is indicated in patients with myelodysplastic syndromes with ≥ 10% of blasts. Azacitidine is recommended in intermediate-2 and high risk IPSS patients who are not eligible for transplantation with minimal number of six cycles to define response. Acute myeloid leukemia like treatment is recommended in fit higher-risk for patients with myelodysplastic syndromes with excess of blasts, good performance status, without substantial comorbidities, and with no poor/very poor cytogenetics/genetics. **Conclusion.** The treatment of fit higher-risk patients should be based on allo-SCT. In patients who are not candidates for transplant hypomethylation treatment is indicated as well as acute myeloid leukemia like treatment in selected patients.

Key words: Myelodysplastic Syndromes; Thearapeutics; Risk Factors; Risk Assessment; Treatment Outcome; Transplantation, Homologous;

Sažetak

Uvod. Mijelodisplastični sindrom predstavlja grupu klonskih bolesti hematopoezne stem-ćelije koju karakterišu: citopenija, krvna displazija, neefektivna hematopoeza, specifični genetski poremećaji i povišen rizik od razvoja akutne mijeloidne leukemije. U ovom radu predstavljamo pregled i preporuke za lečenje mijelodisplastičnog sindroma visokog rizika ispred Aktiva za mijelodisplastični sindrom Srpskog lekarskog društva. **Materijal i metode.** Pregled literature je obavljen korišćenjem različitih bibliografskih baza podataka: *Google Scholar, MEDLINE* i *Kobson*. Preporuke za lečenje mijelodisplastičnog sindroma visokog rizika predstavljaju mišljenje eksperata na osnovu pregleda literature i savremenih preporuka za lečenje mijelodisplastičnog sindroma visokog rizika. **Preporuke.** Mijelodisplastični sindrom visokog rizika se utvrđuje kod obolelih sa IPSS-R skorom > 3,5. Alogena transplantacija matičnih ćelija hematopoeze preporučuje se kod *fit* bolesnika sa mijelodisplastičnim sindromom visokog rizika sa IPSS-R > 3,5 kao i u grupi fit bolesnika nižeg rizika koji pokazuju karakteristike nepovoljnog rizika prema ELN/EBMT internacionalnoj grupi eksperata i *MDS-RIGHT* grupi. Terapija slična za akutne mijeloidne leukemije ili hipometilacijska terapija je indikovana pre transplantacije kod bolesnika sa procentom blasta ≥ 10%. Azacitidin je preporučen u grupi bolesnika sa intermedijernim-2 i visokim IPSS-R rizikom koji nisu kandidati za alogenu transplantaciju uz preporučen minimalan broj ciklusa od šest kako bi se procenio odgovor. Slično lečenje se preporučuje za akutnu mijeloidnu leukemiju u grupi *fit* bolesnika sa mijelodisplastičnim sindromom visokog rizika sa ekscesom blasta, dobrim opštim stanjem, bez značajnih komorbiditeta i bez loše/veoma loše citogenetike/genetike. **Zaključak.** Lečenje *fit* bolesnika sa mijelodisplastičnim sindromom visokog rizika zasniva se na primeni alogene transplantacija matičnih ćelija hematopoeze. Kod bolesnika koji nisu kandidati za transplantaciju indikovano je hipometilacijsko lečenje ili akutne mijeloidne leukemije slično lečenje kod odabranih bolesnika.

Ključne reči: mijelodisplastični sindrom; terapija; faktori rizika; procena rizika; ishod lečenja; alogena transplantacija

Introduction

The myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell disorders characterized by cytopenia, dysplasia, ineffective hematopoiesis, recurrent genetic abnormalities, and increased risk of developing acute myeloid leukemia (AML) [1].

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Abbreviations

MDS	– myelodysplastic syndrome
AML	– acute myeloid leukemia
IPSS-R	– Revised International prognostic scoring system
IPSS	– International prognostic scoring system
Allo-HSCT	– allogeneic hematopoietic stem cell transplantation
HCT-CI	– Hematopoietic Cell Transplantation-specific Comorbidity Index
MAC	– myeloablative conditioning
RIC	– reduced intensity conditioning
PS	– performance status
OS	– overall survival
AZA	– azacitidine
CCR	– conventional care group
LDAC	– low dose cytosine arabinoside

The incidence of MDS is about 3 to 5 cases per one hundred thousand population/year [1, 2]. The median age is above 70 years, with predominance of male patients [1–3].

The MDS patients have heterogeneous clinical course, from patients with slight, stable macrocytic anemia to patients with severe, life threatening pancytopenia and high risk of transformation to AML [1]. The revised International prognostic scoring system (IPSS-R) is a prognostic model based on the level of cytopenia, percentage of blasts in bone marrow and cytogenetics [4]. IPPS-R divide MDS patients in five prognostic groups: low (very low and low risk), intermediate risk, and high risk (high and very high risk) [4]. It has shown better predictive power in comparison with the International prognostic scoring system (IPSS) [4–8].

In this paper, we present the review of literature and recommendations for the treatment of higher-risk MDS on behalf of the Serbian MDS group.

Material and methods

A literature review was conducted using the following bibliographic databases: Google Scholar, MEDLINE and Kobson. The recommendations are based on expert opinion established on review of literature and contemporary recommendations for treatment of higher-risk MDS.

Definition of higher-risk MDS

How to define higher-risk MDS using the IPSS-R is still a matter of debate. Considering the International prognostic scoring system (IPSS) the definition of higher-risk MDS is clear. Intermediate-2 and high-risk groups belong to high-risk MDS, low risk and intermediate-1 risk group make lower-risk MDS [9–11]. Higher-risk MDS is defined by Pfeilstöcker M et al. as intermediate risk group patients with IPSS-R score > 3.5 as well as high/very high risk group [12]. This criterion is accepted and recommended by the National Comprehensive Network (NCNN) and British Society of Hematology (BSH) guidelines [9, 13].

Recommendations

Higher-risk MDS should be defined in patients from intermediate IPSS-R risk group with IPSS-R score > 3.5 as well as in patients from high and very high IPSS-R risk group.

Allogeneic hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only curative MDS treatment [14]. However, the Allo-HSCT is a type of treatment associated with high toxicity and transplant related mortality [14]. MDS is a disease of the elderly with frequent comorbidities among the patient population. Therefore, transplantation is not conducted in the great majority of patients [14]. The general recommendation in MDS is to delay transplant in lower-risk patients and conduct transplant as soon as possible in higher-risk patients [15].

Allo-HSCT is recommended according to the EuropeanLeukemiaNet (ELN) and European Bone Marrow Transplant (EBMT) International expert panel in higher-risk MDS (high and very high risk IPSS-R) as well as in lower-risk (very low, low an intermediate risk IPSS-R) good performance (fit) patients with poor risk features: (very) poor risk cytogenetic/molecular characteristics, persistent blast increase (> 50% increase from base line or with > 15% BM blasts), life threatening cytopenia (neutrophil counts < 0.3 x 10^9/l, platelet counts < 30 x 10^9/l), high transfusion intensity ≥ 2 units/month for 6 months, and progression to higher-risk IPPS-R group [14, 16]. The MDS-RIGHT group accepted the same recommendations and added a poor risk feature defined by drop of platelets > 25% during six months after diagnosis [17–19). MDS right group defined patients fit for transplant with Karnofsky score ≥ 70, Hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score < 3 [17, 20].

The new international prognostic scoring system-molecular (IPSS-M), based on the integration of IPPS-R and next generation sequencing results, has recently been published [21]. It represents an improvement in comparison with IPSS-R [21].

The failure of non-transplant strategies (at least one line of treatment) is considered as an indication for transplant in lower-risk MDS [9, 14, 22]. The percentage of blasts in bone marrow before transplantation above 5% is considered as a prognostic factor in allo-HSCT [23]. Expert opinion is that AML-like treatment or hypomethylation treatment is indicated before transplantation in patients with bone marrow blasts above 10% (MDS EB 2).

Patient age is not a limiting factor for transplantation [14, 23]. It is possible to perform allo-HSCT in selected patients over 70 years who have good performance status and low Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) score [24]. The choice of condition regimen, myeloablative conditioning (MAC) vs. reduced intensity conditioning (RIC) depends on age, performance status (PS), HCT-CI and disease status [10, 13, 14]. In younger MDS patients with good PS and low HCT-CI, MAC regimen is recommended; otherwise, RIC regimen is indicated [13, 14]. The choice of donor is

generally as in AML. The first choice is a sibling donor, then matched unrelated donor, then, haploidentical donor/umbilical cord transplants [13, 14].

Recommendations

Allo-HSCT is recommended in fit higher-risk patients with IPSS-R > 3.5 as well as in fit lower-risk patients with poor risk features according ELN/EBMT International expert panel and MDS right group.

Allo-HSCT should be considered in lower-risk MDS patients after the failure of at least one line of non-transplant treatment.

We recommend the definition of "fit" for transplant patients in cases with Karnofsky score ≥70, hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score <3.

AML-like or hypomethylation treatment before Allo-HSCT is indicated in MDS patients with ≥10% of blasts.

Hypomethylating treatment

Hypomethylating treatment is an alternative treatment to AML-like therapy. It is not a curative treatment; however, it could prolong survival even in MDS cases with poor cytogenetics [25]. The AZA001 study reported that median Kaplan-Meier overall survival (OS) was 24.5 months in the azacitidine (AZA) group compared with 15 months in the conventional care group (CCR) [25]. In subgroup analysis of AZA001 clinical study in patients above 75 years, AZA significantly improved OS vs. CCR and 2-year OS rates were 55% vs. 15% [26]. Azacitidine was recommended as the treatment of choice in patients aged ≥ 75 years with good performance status and higher-risk MDS [26]. However, in practice, the results from AZA001 study were not confirmed. Median survival in patients who received azacitidine was 12 to 13 months, according to results from Spanish, French and Canadian registry, which is clearly a shorter survival comparing with survival in the AZA001 study [27–29].

Azacitidine is indicated in intermediate-2 and high risk IPSS patients who are not candidates for allo-HSCT [25]. The dose of azacitidine is 75 mg/m^2 for seven consecutive days, repeated at 28-day intervals [25]. The alternative dosing schedule is 75 mg/m^2 for five days, off treatment for two days, and two further days of treatment (5–2–2 schedule) [30].

Because of the delayed response to azacitidine, the minimal number of azacitidine cycles to define response is six in patients who did not have progression or excess toxicity during treatment [31]. Azacytidine treatment should be continued until disease progression [9, 10, 13, 31].

Bone marrow examination is required before treatment and after six months of azacitidine treatment [13, 31].

Recommendations

Azacitidine is recommended in intermediate-2 and high risk IPSS patients who are not eligible for transplantation.

The recommended dose is 75 mg/m^2 for seven consecutive days, repeated at 28-day intervals.

The alternative dosing schedule is 75 mg/m^2 for five days, off treatment for two days, and two further days of treatment.

To define response, the minimal number of azacitidine cycles is six.

In the case of response, azacytidine should be continued until loss of response/progression of disease.

Low dose chemotherapy

Low dose chemotherapy is generally not recommended for treatment of higher-risk MDS because of lack of evidence of beneficial influence on survival or risk of AML progression.

Low dose cytosine arabinoside

Low dose cytosine arabinoside (LDAC) has shown inferior survival in comparison with azacytidine in the AZA001 trial [25]. However, in an early randomized study with low dose cytosine arabinoside, there was a 32% overall response rate to a single cycle with 11% complete and 21% partial responses [32]. The average duration of response was 5.9 months, with a range of 1.4–33.5 months [32]. There is no influence on overall survival and transformation to AML. Mortality associated with hematological toxicity is quite high, up to 19% [33, 34]. Low bone marrow cellularity, absence of ring sideroblasts, <2 chromosomal aberrations and normal platelets numbers were predictors of a favorable response [34].

Melphalan

Low dose melphalan (2 mg/day) has shown a response rate of 30% in higher-risk MDS [35–37], which was associated with normocellular or hypocellular bone marrow and with absence of complex cytogenetic anomalies. Toxicity of treatment was low [35–37].

Recommendations

LDAC could be given in selected group of patients with hypocellular/normocellular bone marrow, excess of blasts, without thrombocytopenia and chromosomal aberrations.

LDAC dose could be 20mg twice a day subcutaneously, for ten days. In the case of response, the LDAC treatment could be continued.

Melphalan treatment could be given in selected patients with higher-risk MDS, hypocellular /normocellular bone marrow and normal cytogenetics.

Melphalan dose is 2 mg/day until response (usually 8 weeks) or progression.

AML like treatment

Intensive chemotherapy which is used for treatment of AML (AML-like treatment) could be the treatment of choice for patients with higher risk MDS and excess of blasts, as well as for those patients who transformed to AML [9, 10, 13, 38–41). This type of treatment is associated with substantial toxicity and early mortality [9, 10, 13, 38–40]. The level of complete remissions is from 40% to less than

Table 1. Other MDS drugs and non-approved treatment in higher-risk MDS
Tabela 1. *Drugi lekovi u MDS-u lekovi koji nisu odobreni za lečenje u MDS-u visokog rizika*

Drug name *Ime leka*	Mechanism of action *Mehanizam dejstva*	Overall response rate IWG 2006 criteria *Ukupan procenat pozitivnog odgovora na terapiju IWG, 2006*	Bibliography *Bibliografija*
Decitabine *Decitabine*	Hypomethylation agent *Hipometilacijski lekovi*	17%	Kantarjian et al. Cancer 2006 (42)
Venetoclax + azacitidine/*Venetoklaks + azacitidin*		70% 50% 62% 50%	Wei AH et al. Blood 2019 (43) Zeidan AM et al. Blood 2019 (44) Desikan SP et al. HemaSphere 2022 (45) Minarik L et al. HemaSphere 2022 (46)
CPX-351	a liposomal encapsulation of cytarabine and daunorubicin *Lipozomalna inkapsulisani blik citarabina i daunorubicina*	71%	Peterlin P et al. Blood 2021 (47)

60% [38–40]. It is recommended in patients with good performance status, without substantial comorbidities and without poor prognostic cytogenetics (e.g., complex anomalies, -7, biallelic p53 mutation) [40, 41]. AML-like treatment could be conducted in patients who are not candidates for transplantation or in patients with excess of blasts >10% who are candidates for transplantation in order to achieve remission before transplantation [9, 10, 13, 41]. The patient could be treated with up to two induction cycles, and up to two consolidation cycles.

Recommendations

AML-like treatment is recommended in fit higher-risk MDS patients with excess of blasts, good performance status, without substantial comorbidities, and with no poor cytogenetics/genetics (e.g., del [7], complex anomalies, biallelic p53 mutation).

AML-like treatment could be conducted before allo-HSCT in patients with >10% of blasts.

Other MDS drugs and non-approved treatment in higher-risk MDS

There are other drugs which could be considered in treatment of higher-risk MDS **(Table 1).** Decitabine, a hypomethylation agent, has shown some activity in higher-risk MDS, however, without clear influence on survival and risk of AML transformation [42]. Venetoclax is another drug with substantial efficacy in combination with azacytidine in several small early phase studies [43–46].

CPX-351, a liposomal encapsulation of cytarabine and daunorubicin, has shown a 71% response rate in higher-risk MDS [47).

These agents could not be recommended for treatment yet, because there is insufficient evidence that they modify the course of the disease.

Algorithm of higher-risk MDS treatment

The algorithm of treatment of higher-risk MDS patients is given in **Figure 1.**

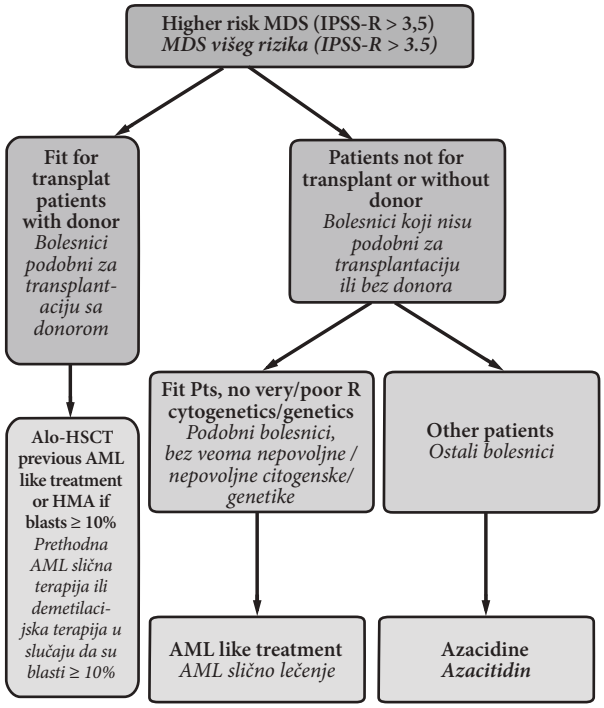

Figure 1. The algorithm of treatment of higher-risk MDS patients
Slika 1. *Algoritam lečenja obolelih od mijelodisplastičnog sindroma visokog rizika*

Conclusion

The treatment of fit higher-risk patients should be based on allogeneic hematopoietic stem cell transplantation in fit patients, with previous acute myeloid leukemia-like or hypomethylation treatment in the case of ≥10% of blasts. In patients who are not candidates for transplant, hypomethylation treatment is indicated or acute myeloid leukemia-like treatment in selected patients.

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TRANSPLANTATION OF HEMATOPOIETIC STEM CELLS
TRANSPLANTACIJA MATIČNIH ĆELIJA HEMATOPOEZE

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POOR GRAFT FUNCTION – CAUSES AND POTENTIAL SOLUTIONS

SLABOST KALEMA – UZROCI I POTENCIJALNA REŠENJA

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Summary

Introduction. Poor graft function is one of the most severe complications after allogeneic hematopoietic stem cell transplantation, which manifests as pancytopenia/cytopenia in the blood count, with the presence of complete or incomplete donor chimerism. There are three entities of graft weakness: 1. poor graft function: pancytopenia with complete donor chimerism, 2. graft failure: pancytopenia with incomplete, i.e., mixed donor chimerism and 3. graft rejection: progressive decline of donor chimerism. **Definition.** Poor graft function is diagnosed as pancytopenia (hemoglobin < 70 g/L, absolute neutrophil count < 0.5 x 10^9/L, platelets < 20 x 10^9/L) for 3 consecutive days from D+28, excluding the presence of severe graft versus host disease and relapse, with complete donor chimerism in poor graft function, and incomplete in graft failure. **Risk factors and therapeutic principles.** The most common risk factors for poor graft function are a small dose of CD34+ hematopoietic stem cells in the transplant, graft versus host disease, cytomegalovirus infection, the presence of donor-specific antibodies, high serum ferritin, i.e., iron overload, as well as splenomegaly. Pathogenetic mechanisms in the development of poor graft function are still not fully elucidated. The role of the microenvironment of the patient's bone marrow is also important, as well as disorders of the immune system Therapeutic options for overcoming this complication include using selected "stem cell boost", mesenchymal stem cells, and newer medical agents (N-acetyl cysteine, atorvastatin, thrombopoietin receptor agonists). **Conclusion.** The type of poor function of the graft is defined in relation to the percentage of donor chimerism, and is necessary for planning further treatment strategy.

Key words: Hematopoietic Stem Cell Transplantation; Graft Rejection; Risk Factors; Pancytopenia; Chimerism; Therapeutics; Prognosis; Antigens, CD34

Sažetak

Uvod. Slabost kalema tj. transplantata je jedna od najtežih komplikacija nakon alogene transplantacije matičnih ćelija hematopoeze, koja se ispoljava kao pancitopenija/citopenija u krvnoj slici, uz postojanje potpunog ili nepotpunog donorskog himerizma. Postoje tri entiteta slabosti kalema, a to su: 1. slaba funkcija kalema (engl. *poor graft function*) kada postoji pancitopenija uz potpuni donorski himerizam, 2. pancitopenija sa nepotpunim tj. mešovitim donorskim himerizmom (engl. *graft failure*) i 3. odbacivanje kalema uz progresivni pad donorskog himerizma. **Definicija.** Slabost kalema se dijagnostikuje kao pancitopenija (hemoglobin < 70 g/L, apsolutni broj neutrofila < 0,5 x 10^9/L, trombociti < 20 x 10^9/L) tri dana za redom od D + 28, pri isključenju teškog oblika bolesti kalema protiv domaćina (engl. *graft versus host disease*) i relapsa, sa potpunim donorskim himerizmom kod PGF-a, a sa nepotpunim kod *graft failure.* **Faktori rizika i terapijski principi.** Najčešći faktori rizika za nastankak slabosti kalema su mala doza CD34$^+$ matičnih ćelija hematopoeze u transplantatu, bolest kalema protiv domaćina, infekcija citomegalovirusom, prisustvo donor-specifičnih antitela, visok serumski feritin tj. opterećenje gvožđem, kao i splenomegalija. Patogenetski mehanizmi u nastanku slabosti kalema su još uvek nepotpuno razjašnjeni. Pored svih navedenih faktora, značajna je i uloga mikrosredine koštane srži pacijenta, kao i poremaćaji imunskog sistema. Terapijske opcije u prevazilaženju ove komplikacije su primena selektovanog *stem*-ćelijskog busta, mezenhimalnih matičnih ćelia, kao i novijih medikamentnih agenasa (N-acetil cistein, atorvastatin, agonisti trombopoetinskih receptora). **Zaključak.** Definisanje tipa slabosti kalema vrši se u odnosu na procenat donorskog himerizma, a neophodno je zbog planiranja strategije lečenja.

Ključne reči: transplantacija hematopoetskih stem ćelija; odbacivanje kalema; faktori rizika; pancitopenija; himerizam; terapija; prognoza; CD34 antigeni

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the replacement and repopulation of the patient's hematopoietic tissue with donor hematopoietic tissue. It represents the concept of applying a conditioning regimen consisted of chemotherapy+/-radiotherapy combined with immu-

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Abbreviations

Allo-HSCT	– allogeneic hematopoietic stem cell transplantation
CMV	– cytomegalovirus
CXCL12	– chemokine stromal cell-derived factor-1
CXCL4	– alpha-chemokine receptor specific for stromal-derived-factor-1
DSA	– donor-specific antibodies
ECs	– endothelial cells
EPO	– erythropoietin
G-CSF	– granulocyte colony-stimulating factor
GPI-AP	– glycosyl phosphatidyl inositol anchored protein
GvHD	– graft versus host disease
GvL	– graft versus lymphoma
HSC	– hematopoietic stem cell transplantation
IGFBP-1	– insulin-like growth factor–binding protein
MSCs	– mesenchymal stem cells
NAC	– N-acetyl-cysteine
PGF	– poor graft function
RANTES	– regulated on activation, normal T cell expressed and secreted
ROS	– radical oxygen species
SCB	– stem cell boost
T regs	– T regulatory lymphocytes
Tc	– T cytotoxic lymphocytes
Th	– T helper lymphocytes
TPO	– thrombopoietin

nosuppression, after which donor hematopoietic stem cells (HSCs) are applied to the patient. It is the only curative method for treating high-risk malignant hematological disorders, and some non-malignant diseases of the hematopoietic system, such as aplastic anemia. The majority of patients recover after allo-HSCT with reconstitution of allogeneic hematopoiesis, but a certain percentage experience the appearance of so-called graft failure (GF), which is caused by the rejection of the graft (graft) with a decline in donor chimerism, or poor graft function (PGF) occurring with complete donor chimerism [1].

There are three entities of graft weakness, namely: 1. poor graft function-PGF, when there is pancytopenia with complete donor chimerism, 2. graft failure-GF, which presents as pancytopenia with incomplete, i.e. mixed donor chimerism, and 3. graft rejection with a progressive decline of donor chimerism.

The appearance of mixed chimerism of donor cells and patient (recipient) cells may reflect on the initial rejection of the graft. Unlike graft rejection, 100% donor chimerism is present in PGF, i.e., the presence of exclusively donor cells in the patient's bone marrow and peripheral blood. For diagnosing PGF, a clear definition of diagnostic criteria is required, but there are still some disagreements [1].

Newer studies define PGF depending on the severity of cytopenia in at least two cell lines, which means that: 1. Absolute neutrophil count (ANC) $\leq$ 0.5 x 10^9/L; 2. Platelets (PLT) $\leq$ 20x10^9/L; and hemoglobin (Hb) $\leq$ 70 g/L in at least 3 consecutive days after D+28 of allo-HSCT, or that transfusion support is required, with hypoplastic bone marrow, and with the prior exclusion of GvHD or disease relapse. Primary PGF represents a failure to achieve engraftment, while secondary PGF represents a loss of initial engraftment. The therapeutic response is worse in primary then in secondary PGF [1]. It is a potentially life-threatening complication given the persistent cytopenia, the constant need for transfusions, and the present risk of recurrent infections. The incidence of PGF is about 5-27%, with a growing tendency, considering the expansion of haploidentical transplants [2]. The mechanism of the formation of PGF has not been fully clarified, but the microenvironment of the bone marrow certainly plays a significant role in its formation. In the therapeutic approach, the selected "stem cell boost" (SCB), infusion of mesenchymal stem cells and new therapeutic principles including atorvastatin, N-acetyl-cysteine, eltrombopag are important [1].

Risk factors for poor graft function

Risk factors for the occurrence of PGF are a low dose of CD34+ cells in the transplant, cytomegalovirus (CMV) infection, GvHD, the presence of donor-specific antibodies (DSA), iron overload and splenomegaly. Also, CMV infection and GvHD are more often associated with secondary PGF vs. primary [3].

An analysis of two recent studies found that a dose of CD34+ cells of 5.5 x 10^6/kg body mass compared to a dose of 2.2 x 10^6/kg body mass was associated with a lower risk of developing PGF (2.89% versus 5.6%; $p = 0.015$). Zao et al. have determined that the dose of CD34+ cells < 5 x 10^6/kg body mass may represent an independent risk factor for developing primary PGF in multivariate logistic regression analysis [2].

Also, the presence of donor-specific antibodies (DSA) in the recipient's blood is associated with the occurrence of PGF. These antibodies present in the recipient's blood are directed towards the donor's HLA antigens. The presence of DSA is associated with a 10-fold increased risk of graft rejection in haploidentical allo-HSCT [1].

DSA quantification is therefore necessary before every haplo HSCT and their value > 1000 MFI (mean fluorescence intensity) is the cut-off for this type of transplantation according to EBMT (European Blood and Marrow Transplantation) guidelines [4]. It is undoubtedly necessary to avoid such DSA-positive donors if possible

Other risk factors for the development of PGF include iron overload, the value of which follows the value of the serum ferritin, as a biological marker. If ferritin is $\geq$ 2000 ng/mL, it represents an independent risk factor for developing primary PGF and poor overall survival [2]. The pathophysiological mechanism of PGF formation in iron overload is due to the accumulation of free oxygen radicals that have a suppressive effect on the bone marrow microenvironment and the differentiation of CD34+ HSC. Also, iron is an essential nutritional factor for the development of bacteria and fungi, leading to a tendency to infections.

A risk factor for the development of primary PGF is also splenomegaly, given that the enlarged

spleen destroys hematopoietic cells and thus compromises engraftment. Therefore, entering the allo-HSCT procedure in patients with an enlarged spleen carries a high risk of failure in achieving a stable engraftment. These are especially patients with myeloproliferative neoplasms (myelofibrosis), in which the spleen is significantly enlarged. It was found that craniocaudal spleen diameter > 12 cm and thickness greater than 4 cm is an independent risk factor for developing PGF [2].

Infection with CMV or viral reactivation occurs in 30-70% of allo-HSCT recipients, with a probability of 60-70% if the patient is seropositive. Namely, the risk for CMV infection is the seropositivity of the recipient and the donor, or the seropositivity of the recipient with the seronegativity of the donor. The mechanism of myelosuppression is affected by CMV through infection of stromal cells [5]. Also, the drugs used for treating CMV infection are extremely myelotoxic, and like the virus, they lead to the appearance of PGF [6]. Also, the HHV-6 virus can exert a pronounced myelosuppressive effect and lead to delayed engraftment if it is reactivated in a patient after allo-HSCT. Its effect on hematopoiesis is also strong, similar to CMV, and the probability of reactivation is based on the fact that over 90% of healthy individuals are infected with this virus in childhood, and reactivation occurs in 30-60% of patients undergoing allo-HSCT [7]

GvHD, as the most severe immune-mediated complication after allo-HSCT, also leads to damage to the bone marrow microenvironment. Patients with GvHD gr I-IV have a significantly lower PLT count on D+50 after transplantation [8]

Hematopoietic recovery in patients with PGF and GvHD is associated with the resolution of GvHD.

Other risk factors for PGF are older age, incompatibility in the ABO blood group system, intensity of conditioning regimen, incomplete match in the HLA gene system (9/10) between donor and recipient, etc.

Known facts about the most common mechanisms of poor graft function

HSCs are located in the microenvironment of the bone marrow (hematopoietic niche) and it enables the complex connection of hematopoietic cells and stromal cells. Most HSCs reside in perivascular spaces. Perivascular stromal cells like mesenchymal stem cells (MSCs) surround them as well as endothelial cells (ECs), adipocytes, magakaryocytes, osteoblasts, macrophages, and regulatory T lymphocytes (T regs).

In addition to the mentioned cells, the cytokines, stem cell factor and CXCL12, are vital components of the niches that regulate the homeostasis of HSCs [9]. HSCs survive in relatively hypoxic conditions in the bone marrow [10]. It is known that the bone marrow microenvironment plays a crucial role in the development of PGF, although not all mechanisms have been fully elucidated.

MCs are reduced in number and functionality under PGF conditions and exhibit a higher degree of apoptosis and senescence [11]. On the other hand, there is an intracellular increase of p-p53, p21, and free oxygen radicals in MSCs in patients with PGF. In this way, the possibility of maintaining the functional capacity of hematopoiesis is reduced.

In addition to MSCs, ECs are an important part of the stroma and accelerate the recovery of the bone marrow vascular network, its cellularity, and affect hematopoietic and immune recovery in mouse models [12].

The number of ECs is significantly lower in patients with primary and secondary PGF [13]. In PGF, ECs are not only numerically reduced, but are also dysfunctional, with impaired proliferation, migration and angiogenesis, with a higher degree of apoptosis and the presence of oxygen radicals. An increase in free forms of oxygen radicals affects the depletion of resting CD34+ bone marrow HSCs [14]. Bone marrow niches normally have hypoxic conditions that are key to maintaining HSC functions in terms of survival, cell cycle control and maintenance of metabolism that protects them from oxidative stress [15].

The immune cells of the bone marrow microenvironment also play a key role in the regulation of hematopoiesis. An increase in pro-inflammatory M1 macrophages and a reduction in anti-inflammatory M2 macrophages were found in patients with PGF [16]. The function of bone marrow macrophages (proliferation, migration, and phagocytosis) is also impaired, and not just their number. The function of CD34+ HSCs, in which there is an upregulation of the p38 MAPK signaling pathway, is also impaired. There is also a significantly higher proportion of Th1 and Tc1 lymphocytes that produce IFN gamma, and a decrease in the proportion of Th2 and Tc2 cells that produce IL-4, which leads to a Th1/Th2 and Tc1/Tc2 shift. Also, Th17 and Tc17 cells (which produce IL-17) were significantly increased, as well as the Th17/T reg ratio in PGF. T reg are key cells in the regulation of the immune response. In prolonged thrombocytopenia as part of PGF, IGFBP-1 (insulin-like growth factor–binding protein) and RANTES (regulated on activation, normal T cell expressed and secreted) are increased, and this leads to impaired function of megakaryocytes [17]. In secondary PGF , GPI-AP (glycosyl phosphatidyl inositol anchored protein) leukocytes and HLA-allele-missing leukocytes are increased.

Main therapeutic principles and options they can offer

Standard therapeutic options in the presence of PGF include the administration of hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), infusion of additional donor cells in the form of stem cell boost (SCB) and secondary allo-HSCT.

The hematopoietic growth factors G-CSF and EPO increase the number of ANC and the Hb value, but for a short time, requiring frequent therapy repetition, which leads to alloimmunization and iron overload due to frequent transfusions.

On the other hand, SCB and secondary allo-HSCT show a higher degree of efficiency. However,

due to the occurrence of GvHD, long-term overall survival is not significantly better after their application [18]. In the course of reduction of acute and chronic GvHD, instead of unselected SCB, CD34+ selected SCB is used [19].

Also, MSCs represent a good therapeutic option due to their own lower immunogenicity compared to HSCs.

The advantage of CD34+ SCB is that a new conditioning regimen, which is necessary in secondary allo-HSCT, does not have to be used, which in this case carries a higher risk of cumulative tissue and cellular toxicity. The therapeutic response rate and recovery from trilineage cytopenia were 83% and 75% in the study by Stasia et. al. regardless of donor type [20]. The procedure is also safe, with a low risk of developing aGvHD grade 2, without worsening the previous cGvHD. There was no significant degree of toxicity related to the use of CD34+ SCB, and with this method, with immunomagnetic selection, T lymphocytes are depleted as much as possible. This is also achieved by positive immunomagnetic selection of CD34+ HSCs besides depletion of T lymphocytes.

Obtaining SCB always requires the donor's willingness to undergo new apheresis after the secondary mobilization process, and in that act, it is necessary to take enough cells and freeze a part of them as a hematopoietic "rescue". They can be thawed and used in repeated application as required by the patient. By comparing the so-called live and cryopreserved cells, which were subsequently thawed and administered, no differences were found in the response rate. Using the combination of G-CSF and plerixafor, antagonist of CXCR-4 receptor on HSCs, increases cell yield of CD34+ HSC, compared to the use of G-CSF alone [21].

Considering that PGF is basically the dysfunction of MSCs in the bone marrow, their application can be an effective treatment strategy [1].

Also, MSCs are significantly less immunogenic and do not need to be collected from the original or previously used donor. It was observed that overall survival after the application of MSCs in the treatment of PGF is about 70%, without significant adverse events [1]. Still, the results related to the application of MSCs were obtained on a small series of patients. In addition to successful hematopoietic recovery, the presence of viral, bacterial and fungal infections was verified in more than half of the patients [22].

New treatment strategies - do they offer better results?

New drugs have shown favorable effects in the treatment of PGF. Thus, atorvastatin, which is used in the treatment of dyslipidemia, improves the function of endothelial progenitor cells (EPCs), both quantitatively and qualitatively in vitro studies [13].

N-acetyl-cysteine (NAC) in murine models improves the engraftment of HSCs by reducing the level of free oxygen radicals (ROS) [23]. Clinical studies have shown that NAC ameliorates the impairment of the function of bone marrow ECs in patients with PGF [24]. Thus, in 35 patients with PGF treated with NAC, there was a reconstitution of bone marrow ECs and CD34+ HSC, which were initially < 0.1%, with a consecutive decrease in the incidence of PGF. Eltrombopag, a thrombopoietin receptor (TPO) agonist, stimulates the TPO receptor c-mpl, both on megakaryocytes and HSCs. This way, it leads to the production of megakaryocytes and platelets and is used for the treatment of aplastic anemia and post-transplantation thrombocytopenia. In treating secondary PGF, it leads to remission in 10/12 patients [1].

Iron chelators such as deferasirox have also been shown to improve the hematopoietic response in some patients with aplastic anemia and acute leukemia.

Olivetti et al. showed that in a patient with aplastic anemia and PGF after allo-HSCT, complete hematological recovery occurs after the application of deferasorox as a chelator [25].

Conclusion

PGF is a severe complication after allo-HSCT and carries a high mortality rate. Patients with PGF have an inferior rate of survival when left untreated. The most important risk factors for its occurrence are a low dose of CD34+ HSC, the presence of DSA, CMV infection, GvHD, iron overload, and splenomegaly. All pre-transplantation risk factors should be considered in order to prevent the occurrence of PGF. Disturbances at the level of bone marrow microenvironment play a key role in pathogenesis, so therapy is aimed at improving the bone marrow microenvironment and hematopoietic recovery. This led to the development of new therapeutic strategies. Fresh or cryopreserved CD34+ SCB without preconditioning is used as a safe and effective treatment strategy. MSCs can improve the prognosis of PGF, but also increase the risk of infections. Furthermore, new therapeutic options such as atorvastatin, NAC, eltrombopag, deferasirox show a good degree of tolerance and good efficacy. So far, all studies have been conducted on a small number of subjects, and some studies have been conducted only in vitro. Therefore, future prospective randomized, well-controlled studies will be necessary to confirm the results so far.

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PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN SERBIA – 25 YEARS OF EXPERIENCE

TRANSPLANTACIJA MATIČNIH ĆELIJA HEMATOPOEZE KOD DECE U SRBIJI – 25 GODINA ISKUSTVA

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Summary

The first allogeneic identical sibling donor hematopoietic stem cell transplantation at the Institute for Mother and Child Health Care of Serbia "Dr. Vukan Čupić" (Institute) was performed in the seventies of the last century. Almost twenty years later, the first allogeneic transplant from sibling haploidentical donor was performed in an infant suffering from severe combined immunodeficiency. After several years of efforts, the bone marrow transplant unit was opened in April 1997 at the Institute. Over the 25 years, the transplant team performed 360 hematopoietic stem cell transplantations, 175 autologous and 185 allogeneic (103 from identical sibling donor, 43 from haploidentical sibling donor, 36 from matched unrelated donor, 2 from identical sibling cord blood and 1 from unrelated identical cord blood).

Key words: Hematopoietic Stem Cell Transplantation + history; Bone Marrow Transplantation + history; Maternal-Child Health Services + istorija; Oncology Service, Hospital + history; Serbia; Child; History, 20th Century; History, 21st Century

Sažetak

Prva alogena transplantacija matičnih ćelija hematopoeze od srodnog davaoca u Institutu za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić" urađena je sedamdesetih godina prošlog veka. Skoro dvadeset godina kasnije urađena je prva alogena transplantacija od srodnog delimično podudarnog davaoca kod odojčeta koje je bolovalo od teške kombinovane imunodeficijencije. Posle višegodišnjih napora, u Institutu za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić" otvoren je sterilni blok aprila 1997. godine. U proteklih 25 godina transplantacioni tim je uradio 360 transplantacija i to 175 autolognih i 185 alogenih (103 od identičnog srodnog davaoca, 43 od srodnog delimično podudarnog davaoca, 36 od nesrodnog podudarnog davaoca, dve od identične srodne krvi pupčanika i jedna od nesrodne identične krvi pupčanika).

Ključne reči: Hematopoietic Stem Cell Transplantation + history; Bone Marrow Transplantation + history; Maternal-Child Health Services + istorija; Oncology Service, Hospital + history; Serbia; Child; History, 20th Century; History, 21st Century

For more than 60 years, hematopoietic stem cell transplantation (HSCT) has been an important form of treatment for numerous congenital and acquired diseases, and for some it is the only possible one. The first HSCTs in human medicine were performed in 1957 (Thomas Donald et al.) for patients suffering from leukemia, and two years later Prof. Georges Mathé et al. performed allogeneic HSCTs in patients irradiated in the Vinca accident. Almost ten years later, the first HSCTs were performed in children suffering from severe combined immunodeficiency and Wiskott-Aldrich syndrome [1-3].

In the first ten years of HSCT in human medicine, the most common indications were refractory malignant diseases of the hematopoietic system or relapses, aplastic anemia and congenital immunodeficiencies. In this early period of transplantation medicine, most transplanted patients were in terminal phases of the disease and died before adequate evaluation. The first successful transplants were performed in patients with congenital immunodeficiencies in 1968/69 [3].

Today, as a result of new knowledge in immunology, molecular biology, better supportive therapy, new drugs, better understanding, prevention and treatment of graft-versus-host disease and other post-transplantation complications, the use of monoclonal antibodies, infusion of donor lymphocytes, mesenchymal stem cells, new conditional regimens and procedures to overcome the mismatch in the HLA System between the patient and the donor, more than 50% transplantee patients can expect 5 years DFS [3, 4].

The first bone marrow transplantation in children in Serbia was performed in the 1970s at the Institute for Mother and Child Health Care of Serbia "Dr. Vukan Čupić" (Institute). Dr Jovan Kezić, the first head of the Hematology and Oncology Department at the Institute and Dr Mirko Mikuška, the first head of the Immunology department, performed the first allogeneic sibling donor HSCT in a girl suffering from acquired aplastic anemia. The bone marrow donor was a brother. Almost twenty years later, in December 1990, at the Institute Dr. Mario Abinun and Dr. Dragana (Makić) Vujić, supported by the help of Prof. Dr. Miomir Malešević

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and Dr. Desa Lilić from the Military Medical Academy in Belgrade and by colleagues from London, performed the first haploidentical HSCT in a nine-month-old infant suffering from severe combined immunodeficiency. It was the first haploidentical HSCT in ex Yugoslavia and the region [4, 5].

Doctors of the Hematology-oncology Department (Dr. Jovan Kezić, Dr. Sofija Aleksandrović-Boberić, Dr. Nada Rašović-Gvozdenović, Dr. Petar Ivanovski) and the Immunology Department (Dr. Mirko Mikuška and Dr. Mario Abinun) of the Institute, supported by the Director (Dr. Božidar Vlajić), in 1986 sent the first request for the creation of a bone marrow transplant unit to the Republic Health Insurance Fund. A long-term "battle" began to obtain consent for the establishment of a bone marrow transplant unit. During 1993/94. the Director of the Institute, Prof. Dr. Miloš Banićević, and Head of the Hematology-Oncology Department, Prof. Dr. Gordana Bunjevački, sent numerous requests to the Ministry of Health, the City Institute for Health Care and the Republic Fund for Health Care and Health Insurance. With these letters, the project "Program for the establishment and operation of the department for intensive therapy and care in the Hematology Service of the Institute for Health Protection of Mothers and Children of Serbia" was submitted. Almost a decade has passed from the time when the first request was filed to the beginning of construction of the bone marrow transplant unit. Construction began in 1994, and three years later, in April 1997, in the newly opened sterile block, the first allogeneic identical sibling donor HSCT was performed on a six-month-old infant suffering from severe combined immunodeficiency. In August 1997, the first autologous HSCT was performed in a girl due to relapsed rhabdomyosarcoma. In this period, part of equipment necessary for the cryolaboratory was provided [4].

Along with the construction of the bone marrow transplantation unit, education of the members of the future transplantation team was organized at the Military Medical Academy in Belgrade and centers in Italy (Trieste, Monza, Genova, Padua), as well as through specialized educational seminars organized by the European Group for Blood and Marrow Transplantation. Standards for bone marrow transplantation in children in Serbia were developed (Dr. Dragana Vujić, Dr. Dobrila Veljković, 1996/1997) [4].

Acting on the initiative of Prof. Dr. Dragana Vujić, with the project team of the Institute for Nuclear Sciences "Vinča" (Prof. Dr. Vujo Drndarević, Danko Đurić, senior advisor and Miodrag Aranđelović), the development of a microprocessor for controlled freezing of hematopoietic stem cells started in 1994. The first tests were carried out in 1995/96. In March 1996, human hematopoietic stem cells from peripheral blood were frozen for the first time. The following year, in July 1997, a microprocessor for controlled freezing of hematopoietic stem cells was put into operation [6, 7].

During the construction of the bone marrow transplantation unit, part of the equipment for the development of the hematopoietic stem cell transplantation program for children in Serbia was provided through the Republic Health Fund and Health Insurance of Serbia [4].

The establishment of a cryobiology laboratory at the bone marrow transplantation unit at the Institute was supported by the Italian humanitarian organization Nuova Frontiera (in 2001), and Gruppo L'Cesvi Espresso provided the equipment necessary for further development of the HSCT program in children in Serbia (in 2002). A bank of fibroblasts and mononuclear cells from patients with congenital diseases was formed within the laboratory for cryobiology [4].

Until 2004, the transplant team of the Institute performed 1-3 HSCTs per year. With the support and assistance of the Ministry of Health of the Republic of Serbia and the Health Insurance Fund of the Republic of Serbia, since 2005, a constant increase in the number of HSCTs performed on an annual basis has been registered. The transplant team performs 22 to 26 transplants per year, which is more than 85% of all transplants performed annually. Additionally, the transplant team worked actively on the introduction of new therapeutic modalities. The first haploidentical HSCT in a newly formed sterile block was performed in 2006 on an infant who had refractory acute leukemia, using pharmacological T depletion of a graft of hematopoietic stem cells. The allogeneic transplant from an unrelated matched donor on an infant suffering from hemophagocytic lymphohistiocytosis was performed in November 2009. It is the first such transplant in Serbia, and the donor was found in the Registry of Voluntary Bone Marrow Donors of Serbia. Four years later, the first matched unrelated donor HSCT from a voluntary donor found in the World Marrow Donor Registry was performed. Alpha, betaTCR/CD19 depletion for haploidentical HSCT was performed in 2012, and thus we became the third pediatric center in Europe to apply this method. The first transplantation of hematopoietic stem cells from umbilical cord blood from an identical related donor was performed at the Institute in 2017. The cord blood sample used at the time was stored in the family cord blood bank, which was founded at the Institute in 2009 with the consent of the Ministry of Health. In December 2017, the first matched unrelated cord blood transplantation was performed on a boy suffering from mucopolysaccharidosis [4, 8–10].

The project on the formation of a public/family cord blood bank with the selfless help of Dr. Momcilo Janković (Ospedale San Gerrardo, Monza, Italy) and the humanitarian organization Gruppo L'Cesvi Espresso (Milan, Italy) was carried out in 2001. The following year, in 2002, the first cord blood sample was collected and frozen during the birth of a brother of a boy who had infant leukemia. At the competition of the Ministry of Health in 2007 for obtaining funds from the budget for development in the field of health care, we submitted the project "Ensuring conditions for hematopoietic stem

cell transplantation in children in Serbia". The project was accepted and its implementation started in 2008. The project provided equipment that enabled the introduction of new diagnostic and therapeutic protocols, and thus further development of hematopoietic stem cell transplantation in children in Serbia. Education of the members of the transplantation team at the Institute was rendered possible. The project envisages the construction of a public/family umbilical cord blood bank and the construction of a new bone marrow transplantation unit with double the capacity in respect to the existing unit. After creating the conceptual, technological and main project for the public/family cord blood bank and the new bone marrow transplantation unit (during 2010 and 2011) and obtaining the necessary permits, the construction of the national public/family cord blood bank began in April 2013. In February 2022, the procedure for obtaining a use permit for a public/family cord blood bank was initiated [4, 9].

As a result of the help and support of Mrs. Svetlana Vukajlović and numerous donors, in 2010, the so-called parent's house within the Institute was opened. It is the first parent's house in Serbia. It is intended for accommodation of children and parents during treatment for hematological-oncological diseases [4].

We participated in scientific research projects, financed by the Ministry of Education, Science and Technological Development. The projects deal with the diagnosis of herpes virus infection after HSCT, infections caused by toxoplasmosis and mesenchymal stem cells of healthy bone marrow donors [11–16].

Since the establishment of the bone marrow transplantation unit in April 1997 until April 2022, the transplant team of the Institute performed 360 HSCTs in children aged 2 months to 237 months (40.1% were under 5 years). 175 patients underwent autologous and 185 allogeneic HSCT. Allogeneic HSCT from identical related donor was performed in 103 patients, haploidentical donor in 43, from unrelated matched donor in 36, 2 transplants using related identical cord blood and 1 from unrelated cord blood. The most common indications for allogeneic HSCT were acute leukemia, congenital immunodeficiency, acquired aplastic anemia, and for autologous HSCT neuroblastoma, Sa Ewing/PNET and lymphomas. Further disease progression after HSCT was the cause of death in 35% of patients, and in 10.3% of patients due to post-transplantation complications.

Since the declaration of the SARS-CoV-2 pandemic, numerous problems arose in organizing HSCT in children, which is why, in accordance with the recommendations of the European Group for Blood and Marrow Transplantation, we froze hematopoietic stem cells from unrelated matched donors. Due to the SARS-CoV-2 infection either in the patients or the donor, the HSCTs were postponed for several patients. From the beginning of the pandemic, organizing the import of stem cells from unrelated matched donors was a big challenge [17].

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HAPLOIDENTICAL DONOR THE DONOR OF CHOICE IN THE TREATMENT OF RELAPSE HODGKIN'S LYMPHOMA

HAPLOIDENTIČNI DONOR DONOR IZBORA U TERAPIJI RELAPSA HIČKINOVOG LIMFOMA

Marija ELEZ[1] and Milena TODOROVIĆ BALINT[2,3]

Summary

Introduction. Modern treatment of Hodgkin's lymphoma has led to a cure in 60-90% of patients. The problem are patients in whom disease relapse occurs, or the disease is refractory to initial therapy. The standard relapse treatment is the application of high-dose chemotherapy with autologous stem cell transplantation. However, about half of patients experience treatment failure after autologous stem cell transplantation. Despite the application of new therapeutic modalities, the disease relapses, so allogeneic stem cell transplantation is the method of choice in further treatment. **Haploidentical transplantation - yes/no.** The donor for an allogeneic transplant can be related or unrelated to human leukocyte associated antigen-matched or haploidentical related. Allogeneic transplantation is used in Hodgkin's lymphoma because of the strong effect of the graft against lymphoma. The dilemmas of whether a haploidentical donor compared to a related/unrelated matched donor are better and when treating of Hodgkin's lymphoma with allogeneic stem cell transplantation are presented in this paper. **Conclusion.** Allogeneic transplantation is still the only potentially curative therapeutic option to treat Hodgkin's lymphoma. In order for the treatment outcome to be as good as possible, it is necessary to precisely define the pre-transplant conditioning, as well as the selection of the donor.. Randomized multicenter studies provide answers to all doubts.

Key words: Hodgkin Disease; Transplantation, Haploidentical; Transplantation, Homologous; Donor Selection; Treatment Outcome; Recurrence

Sažetak

Uvod. Savremeno lečenje Hočkinovog limfoma dovelo je do izlečenja 60−90% bolesnika sa Hočkinovim limfomom. Problem su bolesnici kod kojih se javlja recidiv bolesti ili je bolest refarakterna na uvodnu terapiju. Standard lečenja relapsa je primena visokodozne hemioterapije sa autolognom transplantacijom matičnih ćelija. Ipak, oko polovine bolesnika doživi neuspeh lečenja nakon autologne transplantacije. I pored primene novih terapijskih modaliteteta, dolazi do relapsa bolesti, te je alogena transplantacija matičnih ćelija metoda izbora u daljem lečenju. **Haploidentična transplantacija: da/ne.** Donor za alogenu transplantaciju može biti srodni ili nesrodni sistem leukocitnih antigena podudarni ili haploidentični srodni. Alogena transplantacija se kod Hočkinovog limfoma primenjuje zbog postojanja snažnog efekta kalema protiv limfoma. Dileme da li je haplo donor u odnosu na srodnog/nesrodnog podudarnog donora bolji i kada u lečenju Hočkinovog limfoma alogenom transplantacijom prikazane su u ovom radu. **Zaključak.** Alogena transplantacija je još uvek jedina potencijalno kurativna terapijska opcija u lečenju Hočkinovog limfoma. Kako bi ishod lečenja bio što bolji, potrebno je precizno definisati pretransplantcionu pripremu kao i izbor donora. Odgovore na sve nedoumice pružaju randomizovane multicentrične studije.

Ključne reči: Hočkinova bolest; haploidentična transplantacija; alogena transplantacija; izbor donora; ishod lečenja; relaps

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents a potentially curative option for the treatment of patients with primary resistant or relapsed chronic lymphoproliferative diseases, and the curative potential is achieved through an immune-mediated graft-versus-lymphoma (GvL) effect. The data on the reduced incidence of relapse after allo-HSCT in patients with Hodgkin's lymphoma (HL) compared to autologous hematopoietic stem cell transplantation (auto-HSCT) (6-29% vs. 35-69%) is the best evidence for the existence of the GvL effect [1]. Favorable therapeutic response in this group of patients is achieved in 30-56% of cases [2]. However, post-transplantation complications leading to high non-relapse mortality (NRM) represent a limiting factor for the broader application of haploidentical HSCT in the treatment of these patients, especially in the era of new drugs such as monoclonal antibodies and checkpoint inhibitors [3–5].

Haploidentical hematopoietic stem cell transplantation - YES

New agents lead to high overall response rates, but long-term follow-up is still discouraging. Namely, only 9 out of 102, i.e. 11.3% of patients with R/R HL treated with brentuximab vedotin, have a five-year disease-free survival [6]. Also, the period until a new relapse (EFS) in such patients treated with pembrolizumab, is only 16.5 months [7]. According to current EBMT rec-

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Abbreviations

Allo-HSCT – allogeneic hematopoietic stem cell transplantation
Auto-HSCT – autologous hematopoietic stem cell transplantation
CIBMTR – Center for International Blood and Marrow Transplant Research
Cy – cyclophosphamide
EBMT – European Group for Blood and Marrow Transplantation
EFS – event free survival
GvHD – graft versus host disease
GvL – graft versus lymphoma
HCT-CI – Hemopoietic cell transplantation – comorbidity index
HL – Hodgkin lymphoma
NRM – non relapse mortality
OS – overall survival
PET – positron emission tomography
PFS – progression free survival
R/R HL – relapse/refractory Hodgkin lymphoma

ommendations, allo-HSCT from a matched related or unrelated donor is the standard of care in relapse after auto-HSCT. Given the probability of finding HLA-matched donors, alternative donors, such as imperfectly matched unrelated or haploidentical donors, are acceptable options. In this sense, haploidentical HSCT without T-cell depletion, pioneered by John Hopkins and the group from Seattle, stands out in the past ten years. This approach involves a non-myeloablative conditioning regimen with post-transplant cyclophosphamide (Cy) administration, which is accompanied by a low incidence of GvHD and NRM [8–10].

Treatment outcome after haplo-HSCT in R/R HL was analyzed in three large retrospective registry-based analyses and confirmed no difference in overall survival (OS) and survival to progression (PFS) compared with patients treated with related or unrelated allo-HSCT (3-year OS 82% in the haplo HSCT group vs. 83-79% after unrelated allo-HSCT with/without ATG; 3-year PFS is 63% vs. 61-63%) [11]. They came to the same conclusion within the working group for lymphomas at EBMT, as well as in CIBMTR [12, 13]. The results of other uni- or multicenter studies are also encouraging, according to which 3 to 4 - year OS is 54 to 77%, PFS is 38 to 66% with an acceptable NRM of 4 to 26%, which all correspond to the results after allo-HSCT from a matched relative and unrelated donor. A CIBMTR-based report even showed a significantly lower risk of developing chronic GvHD after haploidentical HSCT compared to related HLA-matched allo-HSCT (HR 0.45). The unexpected conclusion of both large registries refers to a significantly lower risk of relapse after haplo-HSCT (HR 0.69-0.74), which was also shown in the Unicenter trial by Burroughs and associates [14]. The incidence of relapse after three years is significantly lower in patients treated with haplo-HSCT compared to related allo-HSCT (13% vs. 62%), regardless of the status of the disease before transplantation [15]. Such results indicate a powerful immune activity and a potent GvL effect.

Considering all the above, haplo-HSCT represents an effective form of treatment for R/R HL and according to the latest EBMT consensus, this approach with post-transplant Cy administration is recommended in cases where a matched family or unrelated donor is not available and urgent HSCT is indicated [16].

Haploidentical hematopoietic stem cell transplantation - NO

Patients with the active disease before transplantation, with a poor general condition and a high comorbidity index (HCT-CI) have an increased risk of various complications after haploidentical HSCT, so these are factors that determine the application of some other therapeutic options in the treatment of R/R HL and have an advantage over forms of HSCT [17]. According to the data of the Italian study group, disease activity according to PET Scan (Deauville score ≥ 4) and HCT-CI ≥ 3 are two crucial parameters for the outcome of haploidentical HSCT in terms of risk of relapse, PFS, NRM and OS, and the conclusion is imposed that in patients with R/R HL, the same is not indicated [18].

New drugs that are expected to improve the therapeutic response in these patients are primarily the already mentioned brentuximab vedotin and checkpoint inhibitors. It remains to be further investigated whether, after their application, it is still indicated to consider some form of allo-HSCT [19–21]. It should be borne in mind that the use of check point inhibitors increases the risk of developing GvHD after allo-HSCT, which is not the case with haplo-HSCT, and is explained by the post-transplantation use of Cy [22].

The strategy for achieving the best possible therapeutic response and possibly curing patients with R/R HL, implies better control of the disease using new drugs or types of HSCT. When we talk about allo-HSCT, which is still the only potentially curative therapeutic option, in order for the treatment outcome to be as good as possible and the complications to be acceptable, it is necessary to precisely define the pre-transplant conditioning and the choice of the donor i.e., the source of HSC. Answers to all doubts should be sought through randomized multicenter studies.

Conclusion

Allogeneic transplantation is still the only potentially curative therapeutic option in the treatment of relapse/refractory Hodgkin lymphoma. Haplo-hematopoietic stem cell transplantation represents the treatment of choice when matched family or unrelated donor is unavailable, and urgent hematopoietic stem cell transplantation is indicated in patients who respond well to previous treatment. In patients with active disease before transplantation, with a poor general condition and a high comorbidity index, there is an increased risk of various complications after haplo-hematopoietic stem cell transplantation, so they are candidates for allo-hematopoietic stem cell transplantation from related/unrelated matched donor. In order to optimize the post-transplant response, it is necessary to precisely define the pre-transplant conditioning, as well as the selection of the donor. Randomized multicenter studies provide answers to all possible doubts.

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INDOLENT LYMPHOMAS AND CHRONIC LYMPHOCYTIC LEUKEMIA
INDOLENTNI LIMFOMI I HRONIČNA LIMFOCITNA LEUKEMIJA

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RESISTANCE TO TARGETED THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

OTPORNOST NA CILJANU TERAPIJU KOD HRONIČNE LIMFOCITNE LEUKEMIJE

Ivana MILOŠEVIĆ

Summary
Targeted therapy with inhibitors of cell signaling pathways and inhibitors of anti-apoptotic molecules significantly improved treatment of chronic lymphocytic leukemia. Inhibitors of Bruton's tyrosine kinase and inhibitors of bcl2 protein showed significant efficacy in either treatment-naïve or relapsed/refractory patients and in patients with poor risk factors. The majority of patients respond to treatment and have durable remissions, but some of them develop resistance, which leads to clinical relapse. The most frequent cause of resistance is mutations on the binding site of targeted molecules, such as Bruton's tyrosine kinase mutations in patients treated with ibrutinib or acalabrutinib, or bcl2 mutations in patients treated with venetoclax. There are also alternative mechanisms that can lead to resistance, such as mutations of another molecule in Bruton's tyrosine kinase signaling pathway, PLCG2, or overexpression of bcl2 protein. These mutations have been detected several months before clinical sings of relapse, and therefore could serve as predictive markers of treatment failure. When resistance to inhibitors of Bruton's tyrosine kinase occurs, treatment with bcl2 inhibitors will be effective in most cases, and vice versa. Other strategies for overcoming resistance to inhibitors of Bruton's tyrosine kinase or bcl2 protein are treatment with PI3K inhibitors, second and third generation Bruton's tyrosine kinase inhibitors, bispecific antiCD3/CD19 antibodies, chimeric antigen receptor T-cells and allogenic stem cell transplantation. Several molecules, which can inhibit or degrade different signaling targets in chronic lymphocytic leukemia cells, are currently under investigation, and they could be effective in patients resistant to inhibitors of Bruton's tyrosine kinase and bcl2 protein.
Key words: Molecular Targeted Therapy; Drug Resistance; Leukemia, Lymphocytic, Chronic, B-Cell; Protein Kinase Inhibitors; Signal Transduction; Mutation

Sažetak
Ciljana terapija inhibitorima intracelularnih signalnih puteva i inhibitorima antiapoptotičkih molekula dovela je do velikog napretka u lečenju hronične limfocitne leukemije. Terapija inhibitorima Brutonove tirozin kinaze i inhibitorima bcl2 proteina je efikasna kod bolesnika koji započinju lečenje, bolesnika sa relapsom bolesti ili refrtakternih na prethodno lečenje, kao i kod bolesnika sa nepovoljnim prognostičkim markerima. Većina bolesnika ima dobar odgovor na terapiju i postiže dugotrajnu remisiju, ali kod nekih može doći do rezistencije na lekove i do kliničkog relapsa. Najčešći uzrok rezistencije je mutacija ciljanog molekula na mestu vezivanja leka, kao što je mutacija Brutonove tirozin kinaze kod pacijenata koji su lečeni ibrutinibom ili akalabrutinibom, ili mutacija bcl2 proteina kod bolesnika lečenih venetoklaksom. Postoje i drugi mehanizmi nastanka rezistencije, na primer, mutacija drugog molekula koji učestvuje u signalnom putu Brutonove tirozin kinaze, PLCG2, ili prekomerna ekspresija bcl2 proteina. Mutacije se mogu otkriti nekoliko meseci pre kliničkog relapsa i njihova detekcija može biti prediktivni marker neuspeha terapije. Kada dođe do rezistencije na inhibitore Brutonove kinaze, najčešće je lečenje inhibitorima bcl2 proteina uspešno, i obrnuto. Ostale strategije za prevazilaženje rezistencije na inhibitore Brutonove tirozin kinaze ili bcl2 proteina su primena kombinovane terapije, inhibitora PI3K, druge ili treće generacije inhibitora Brutonove tirozin kinaze, bispecifičnih antiCD3/CD19 antitela, terapije himeričnog antigena receptora na T-ćelijama i alogene transplantacije matičnih ćelija hematopoeze. U toku su istraživanja nekoliko molekula koji mogu blokirati ili degradirati komponente signalnih puteva u ćeliji hronične limfocitne leukemije i koji bi mogli biti efikasni u lečenju bolesnika rezistentnih na inhibitore Brutonove tirozin kinaze i bcl2 proteina.
Ključne reči: molekularna ciljana terapija; rezistencija na lekove; hronična limfocitna leukemija; inhibitori protein kinaze; sprovođenje signalnim putevima; mutacija

Introduction

The landscape of chronic lymphocytic leukemia (CLL) treatment was significantly changed in the past decade with the discovery of targeted therapy – inhibitors of small molecules that are involved in signaling pathways of the malignant cells. Now, in clinical practice, inhibitors of Bruton's tyrosine ki-

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Abbreviations

CLL – chronic lymphocytic leukemia
BTK – Bruton's tyrosine kinase
bcl2 – B-cell lymphoma 2
PI3K – phosphoinositide 3-kinase
TP53 – tumor protein 53
IgVH – immunoglobulin variable heavy chain gene
BCR – B-cell receptor
PLCG2 – phospholipase C gamma 2
NF-kB – nuclear factor kappa B
AKT – protein kinase B
RAS – rat sarcoma virus
MAPK – mitogen-activated protein kinase
SF3B1 – splicing Factor 3b Subunit 1
MLL2 – histone3-lysine4 N-mono-methyltransferase gene
PCLO – piccolo presynaptic cytomatrix protein
EP300 – E1A binding protein p300
MYC – myelocytomatosis oncogene
ERK – extracellular-signal-regulated kinase
BH3 – B-cell lymphoma 2 homology 3
Bcl-xl – B-cell lymphoma xl
MCL1 – myeloid cell leukemia 1
BFL/A1 – bcl-2-related protein A1
BAFF – B-cell activating factor
APRIL – a proliferation-inducing ligand
LYN – Lck/Yes novel tyrosine kinase
SYK – spleen tyrosine kinase
CART – chimeric antigen receptor T-cells
alloSCT – allogeneic stem cell transplantation
ORR – overall response rate
PFS – progression-free survival
JAK – Janus kinase
PD1 – programmed cell death protein 1
PLK1 – polo-like kinase 1
TOR – target of rapamycin
MEK – mitogen-activated protein kinase kinase
MALT1 – mucosa-associated lymphoid tissue lymphoma translocation protein 1
TMĆH – transplant-MĆH

nase (BTK), inhibitors of bcl2 protein and inhibitors of phosphoinositide 3-kinase (phosphatidilinositol3-kinase, PI3K) are in use, with high efficacy in previously untreated and refractory/relapsed CLL, but also in patients with poor prognostic markers, such as deletion of the chromosome 17, mutation in the tumor protein 53 gene (TP53), unmutated IgVH genes or complex karyotype. Nevertheless, in some patients, treatment fails, or after a good initial response the disease progressed or transformed to more aggressive hematological malignancy – they developed primary or acquired resistance to targeted therapy **(Table 1)**.

Resistance to targeted therapy

Resistance to Bruton's tyrosine kinase inhibitors

Bruton's tyrosine kinase is a part of a signaling pathway in B-lymphocytes that promotes cell differentiation and activation. After B-cell receptor (BCR) stimulation BTK is activated and phosphorylates phospholipase C gamma 2 (PLCG2), which induces activation of NF-κB, AKT, RAS, MAPK, and that promotes cell surviving, proliferation, migration, adhesion and homing. Signaling via BCR and BTK is more intensive in CLL than in normal B cells, which leads to clonal proliferation, rescue of apoptosis and accumulation of malignant cells [1].

Ibrutinib, first class BTK inhibitor, binds to C481 domain of BTK forming covalent, irreversible bond and interrupt BTK signaling pathway [2]. Treatment with ibrutinib is effective in patients with poor prognostic factors (del17, mutTP53, complex karyotype, unmutated genes for IgVH) and in patients previously treated with multiple lines of therapy [3]. However, some patients do not respond to ibrutinib treatment from the start – primary resistance, while some others after good initial response have disease progression – acquired (secondary) resistance. Primary resistance to ibrutinib is present in about 13% of relapsed/refractory CLL patients, but it is more frequent in patients with poor prognostic markers (about 43%). Secondary resistance to ibrutinib develops in 8-13% of CLL patients and in about 80% of cases is caused by the acquired mutations of BTK and PLCG2 [4].

The most common and first discovered BTK mutation that causes resistance to ibrutinib is C481S, which is localized at the binding site for ibrutinib and develops when cysteine is replaced with serine. C481C mutation disrupts ibrutinib irreversible binding to BTK and signaling via BTK is not inhibited, which leads to proliferation of CLL cell and disease activity. Mutation C481S in most cases occurs between the second and fourth year of treatment with ibrutinib (median 34.3 months) [5].

Table 1. The most common mechanisms of resistance to BTK and bcl2 inhibitors
Tabela 1. *Najčešći mehanizmi nastanka rezistencije na BTK i bcl2 inhibitore*

I generation BTK inhibitors *I generacija BTK inhibitora*	BTK mutations/*BTK mutacija* (C481S, C481R, C481F, C481Y) PLCG2 mutations/*PLCG2 mutacija* (L845F, S707Y, R665W)
II generation BTK inhibitors *II generacija BTK inhibitora*	BTK mutations/*BTK mutacija* (C481S, L528W)
III generation BTK inhibitors *III generacija BTK inhibitora*	BTK mutations/*BTK mutacija* (L512, E513G, F517L, L547P)
PI3K inhibitors/*PI3K inhibitori*	Unknown/*Nepoznato*
bcl2 inhibitors/*bcl2 inhibitori*	bcl2 mutations/*bcl2 mutacija* (G101V, D103Y) Overexpression of bcl2/*prekomerne ekspresije bcl2*

Later investigations revealed more mutations in binding BTK domain - C418R, C481F, C481Y, and rare mutations in different position in BTK (R28S, G164D, T316A, T474I/S, R490H, Q516K, L529W, V5371). In about half of resistant cases mutations are present in minor subclones with low variant allele frequencies [6]. Mutations occur more frequently in lymph nodes, and sometimes cannot be detected in peripheral blood. Interestingly, BTK mutation, as well as PLCG2 mutations, can be detected several months before signs of clinical relapse (9-15 months) [7]. About 40% of patients with Richter's transformation also carry BTK mutations which is in most cases detected within 15 months of ibrutinib treatment [8].

Mutation of PLCG2 is another mechanism of ibrutinib resistance and occurs in approximately 12% of cases. Several mutations of PLCG2 are detected – L845F, S707Y, R665W, which enable continuous BCR signaling irrespective of BCR activation. Some patients carry both BTK and PLCG2 mutations [9].

In approximately 20% of patients resistant to ibrutinib, BTK and PLCG2 mutations are not present, with mechanisms of resistance that are not yet fully understood. Multiple changes in resistant CLL cells are detected – deletions of 18p, 17p, mutations of SF3B1, MLL2, PCLO, EP300, abnormal MYC and bcl2, down or up expression of BTK, complex karyotype, epigenetic changes and influence of microenvironment, but their significance in ibrutinib resistance is still unclear [10].

Second generation BTK inhibitors (acalabrutinib, zanabrutinib) covalently bind to BTK at the same domain as ibrutinib. BTK mutations were detected during the treatment with acalabrutinib (C481S, L528W), causing resistance to treatment. Third generation BTK inhibitors (fenebrutinib, vecabrutinib, LOXO-305, ARQ-531, GDC-0851) that non-covalently binds to BTK are in an early phase of clinical studies. BTK mutations which can decrease efficacy of these inhibitors have already been detected (mutations L512M, E513G, F517L, L547P) [11].

Resistance to PI3K kinase inhibitors

Idelalisib and duvelisib are inhibitors of phosphoinositide 3-kinaseβ, which is a part of BCR signaling pathway. Resistance to PI3K inhibitors during treatment occurs, but none of recurrent mutations were detected. Some studies on mouse models suggest that up regulation of genes coding integrin receptor complex or MAPK/ERK can lead to resistance but, so far, precise mechanism of resistance to PI3K inhibitors is unknown [12].

Resistance to bcl2 inhibitors

Venetoclax is BH3 mimetic and selective inhibitor of bcl2 anti-apoptotic protein. Treatment with venetoclax monotherapy or in combination with antiCD20-antibodies is effective in untreated and R/R CLL patients. In patients that developed resistance to venetoclax the most frequent cause are bcl2 mutations, G101V and D103Y, which disrupt venetoclax binding to bcl2. These mutations have variable variant allele frequencies (1.4% do 70%), and expression of variant alleles can increase over time [13]. In most cases, bcl2 mutations were detected 19-36 months after the start of treatment. Bcl2 mutations can be present 25 months before signs of clinical relapse, and 6 months after discontinuation of treatment. Some patients carry both bcl2 mutations that exist in different CLL subclones [14].

Resistance to venetoclax can be induced by overexpression of anti-apoptotic proteins – bcl-XL, MCL and BFL/A1, revealed in lymph nodes and bone marrow. Interaction between CLL cells, nurse-like cell from microenvironment and T cells, and secretion of cytokines and chemokines (BAFF and APRIL), leads to overexpression of pro-survival proteins that neutralizes pro-apoptotic effect of venetoclax [15].

Treatment strategy for patients resistant to targeted therapy

The most important question is how to continue treatment in patients that developed resistance to targeted therapy, especially patients with poor prognostic markers or heavily pretreated patients. Different strategies can be used to overcome resistance to ibrutinib and venetoclax – switching from one drug to another, second and third generation of Bruton's kinase inhibitors, PI3K inhibitors, inhibitors of other BCR signaling pathway components (LYN and SYK inhibitors), inhibitors of different oncogenes, BTK degraders, T-cell activation (bi-specific antibodies, CART) and allogenic stem cell transplantation (alloSCT) **(Table 2)**. Since BTK and bcl2 mutations occur several months before signs of clinical relapse, further investigations are needed to deter-

Table 2. Treatment approach to patients resistant to ibrutinib and venetoclax
Tabela 2. *Terapija bolesnika rezistentnih na ibrutinib i venetoklaks*

Ibrutinib resistance/*Rezistencija na ibrutinib*	Venetoclax resistance/*Rezistencija na venetoklaks*
Venetoclax/*Venetoklaks*	Ibrutinib/*Ibrutinib*
III generation BTK inhibitors/*III generacija BTK inhibitora*	II and III generation BTK inhibitors *II i III generacija BTK inhibitora*
PI3K inhibitors/*PI3K inhibitori*	
Ibrutinib + Venetoclax/*Ibrutinib + Venetoklaks*	
Bi-specific antiCD3/CD19 antibodies/*Bi-specifični anti CD3/CD19 antitela*	
Chimeric antigen receptor T-cells/	
AlloSCT/*Alo TMĆH*	

mine if patients should be tested in regular intervals, and whether the treatment modality has to change immediately after detection of these mutations.

In most countries, ibrutinib and venetoclax are first targeted therapy approved for treatment of chronic lymphocytic leukemia. Since these drugs induce apoptosis of CLL cells trough different mechanisms, switching treatment from ibrutinib to venetoclax and vice versa is effective in resistant patients. Jones et al showed that patients resistant to ibrutinib had 60% overall response rate (ORR) after venetoclax monotherapy [16]. In a study from Lin et al patients resistant to venetoclax were treated with ibrutinib and 69% had 24-month progression free survival (PFS) [17]. In another study (Mato et al) patients resistant to venetoclax were treated with ibrutinib, and ORR was 84% in ibrutinib-naïve patients and 54% among patients which received ibrutinib prior to venetoclax [18].

Efficacy of combination therapy with venetoclax and ibrutinib in standard and high doses in patients resistant to ibrutinib or resistant both to ibrutinib and venetoclax are under investigation. One study showed that patients resistant to ibrutinib and "double" refractory patients had time to treatment failure of 23.7 months and 11.2 months, and overall survival of 47.1 months and 27 months [19]. In some patients, resistance to venetoclax can be reversed if retreatment starts after venetoclax-free period of 10-12 months [20]. In addition, combination of venetoclax and antiCD20-antibodies showed efficacy in some patients previously treated with venetoclax monotherapy [21].

Treatment with PI3K inhibitors, idelalisib and duvelisib, are effective in approximate half of the patients resistant to ibrutinib and venetoclax. Nevertheless, PI3K inhibitors cannot overcome resistance to ibrutinib in patients with PLCG2 mutations [22].

Second-generation inhibitors of Bruton's kinase, acalabrutinib and zanabrutinib, bind to BTK at the same domain as ibrutinib, and patients resistant to ibrutinib due to BTK mutations, are resistant as well to acalabrutinib and zanabrutinib. Nevertheless, second-generation BTK inhibitors are effective in treatment of patients resistant to venetoclax [23]. Third generation BTK inhibitors, fenebrutinib, vecabrutinib, ARQ-531, LOXO-305, are active in the presence of BTK mutations that cause ibrutinib resistance. ARQ-531 is less specific and inhibits additional targets in signaling pathway, and may be active in the presence of PLCG2 mutations. Several clinical trials investigate third-generation BTK inhibitors efficacy in treatment of the patients resistant to first- and second-generation BTK inhibitors or bcl2 inhibitors [24, 25].

SYK and LYN kinase inhibitors are active in the presence of BTK mutations that lead to resistance to ibrutinib, because SYK and LYN are positioned between BCR and BTK in the cell's signaling pathway. Dasatinib and fostamatinib (SYK inhibitors) showed efficacy in treatment of CLL patients, and cerdulinib, SYK and JAK kinases inhibitor, is in early clinical studies.

New strategy in treatment of CLL is to "sensitize" patients T-lymphocytes to recognize and kill malignant cells. Bi-specific antibody CD3/CD19 causes apoptosis of CLL cells from patients with poor prognostic markers and from patients resistant to ibrutinib and venetoclax [26]. CART therapy is effective in treatment of patients resistant to ibrutinib or venetoclax, and "double" refractory patients (ORR 74%-82% after 4 weeks) and some patients had a MRD negativity and durable remissions [27]. Preclinical studies showed that treatment with ibrutinib before CART infusion can decrease cytokine release syndrome and increase efficacy of CART therapy [28].

AlloSCT is effective treatment for patients resistant to targeted therapy, but it is limited to a relatively small number of patients due to older age and multiple comorbidities of the majority of CLL patients [29].

Several small molecules that inhibit or degrade different elements in signaling pathway in CLL cells are under investigation – BTK degraders based on E3 ligase (MT-802), PD-1 inhibitors (pembrolizumab), PLK1 inhibitors (volasertib), and TOR, MEK, MAPK, MALT1 inhibitors. Experimental and preclinical data showed that these agents could be effective in treatment of patients resistant to BTK or bcl2 inhibitors [30].

Conclusion

Targeted therapy with Bruton's tyrosine kinase and B-cell lymphoma 2 inhibitors significantly improved the treatment of chronic lymphocytic leukemia, inducing long-term remissions and prolonged overall survival in the majority of patients, even in patients with poor prognostic factors. Nevertheless, some patients have primary or acquired resistance. The most common cause of acquired resistance are mutations of targeted molecules in the binding domain. Development of these mutations occurs several months before clinical relapse. There are various treatment approaches for patients resistant to Bruton's tyrosine kinase or B-cell lymphoma 2 inhibitors, such as switching the drug, second and third generation Bruton's tyrosine kinase inhibitors, phosphoinositide 3-kinase inhibitors, bispecific antibodies, chimeric antigen receptor T-cells therapy and allogeneic stem cell transplantation.

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CURRENT PRINCIPLES OF FOLLICULAR LYMPHOMA TREATMENT

SAVREMENI PRINCIPI LEČENJA FOLIKULARNOG LIMFOMA

Milica RADOJKOVIĆ

Summary

Over the last 10-15 years, the prognosis of patients with follicular lymphoma has improved, and for the majority of patients, follicular lymphoma is a chronic disease with ten years of overall survival of around 80%. Nevertheless, a certain subset of patients belongs to high-risk follicular lymphoma with early relapses and progressive disease, poor outcomes, and much shorter survival, and there is still no standard approach in the treatment of high-risk follicular lymphoma. Treatment of follicular lymphoma is highly heterogeneous, ranging from a *„watch and wait"* strategy to intensive immunochemotherapy, and needs to be individualized to each patient. An early stage of the disease can be treated with involved-field radiotherapy, which has curative potential. Follicular lymphoma in the advanced stage is still an incurable disease. Standard first-line treatment is a combination of an anti-CD20 antibody (rituximab) and chemotherapy, followed by antibody maintenance. In relapse, treatment with novel anti-CD20 monoclonal antibody-obinutuzumab in combination with chemotherapy. The combination of lenalidomide and rituximab shows good results in patients with relapses and refractory follicular lymphoma, but also as a first-line treatment. An option for elderly patients with comorbidities is rituximab monotherapy. Autologous or allogeneic stem cell transplantation may be an option for a small group of selected patients. In the past decade development of novel targeted agents such as phosphoinositide three kinase inhibitors and immunotherapies (CD20/CD3 bispecific antibody, chimeric antigen receptor T cell therapy) demonstrate the efficiency of chemotherapy-free approach not only for relapsed/refractory patients but also in first-line treatment. A better understanding of the clinical and biological features of follicular lymphoma patients is necessary to improve treatment outcomes in the future.

Key words: Lymphoma, Follicular; Therapeutics; Risk Factors; Treatment Outcome; Molecular Targeted Therapy; Immunotherapy

Sažetak

Poslednjih 10−15 godina prognoza bolesnika sa folikularnim limfomom je poboljšana i folikularni limfom je hronična bolest sa 10-godišnjim preživljavanjem oko 80%. Ipak, izvestan broj bolesnika ima visokorizični folikularni limfom sa ranim relapsima i progresivnom bolešću, lošim ishodom i kratkim preživljavanjem i još nije utvrđen standard lečenja folikularnog limfoma visokog rizika. Terapija folikularnog limfoma je heterogena, individualna za svakog bolesnika i kreće se od principa *watch and wait* do intenzivne imunohemioterapije. Početni stadijum folikularnog limfoma se leči radioterapijom zahvaćenog regiona sa tendencijom izlečenja. Folikularni limfom u odmaklom stadijumu je još uvek neizlečiva bolest. Prva terapijska linija je kombinacija antiCD20 monoklonskog antitela (rituksimab) i hemioterapije, nakon čega se primenjuje terapija održavanja rituksimabom. U lečenju relapsa primenjuje se nova generacija antiCD20 monoklonskog antitela-obinutuzumab u kombinaciji sa hemioterapijom. Primena kombinovane terapije lenalidomidom i rituksimabom pokazuje dobre rezultate kod bolesnika sa relapsnim i refraktarnim folikularnim limfomom, ali i kao prva terapijska linija. Stariji bolesnici sa komorbiditetima se mogu lečiti monoterapijom rituksimabom. Kod manjeg broja dobro odabranih bolesnika se može primeniti autologna ili alogena transplantacija matičnih ćelija hematopoeze. U protekloj deceniji razvoj novih ciljanih agenasa, kao što su inhibitori fosfoinozitid 3 kinaze i imunoterapija (CD20/CD3 bispecifična antitela, himerična antigen-receptorska T-ćelijska terapija) predstavljaju efikasan način lečenja bez primene hemioterapije, ne samo za bolesnike sa relapsom i refrakternom bolešću, već i kao prva terapijska linija. Za poboljšanje ishoda lečenja folikularnog limfoma u budućnosti neophodno je unapređenje znanja o kliničkim i biološkim karakteristikama bolesti.

Ključne reči: folikularni limfom; terapija; faktori rizika; ishod lečenja; ciljana terapija; imunoterapija

Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma accounting for 70% of cases in Western countries [1]. FL is a biologically and clinically heterogeneous disease. The Hallmark of FL occurring in more than 85% of patients is translocation t(14;18) and the presence of bcl2/IgH gene rearrangement, which results in overexpression of the bcl2 protein that blocks apoptosis. Nevertheless, the presence of translocation t(14;18) is insufficient for lymphomagenesis and additional genomic aberrations are required. Most FL carries multiple epigenetic lesions and mutations on histone post-translational modifying genes such as enhancers of zeste homologue 2 (EZH2) [2]. Recent studies suggest that chromatin-modifying gene mutations and altered function of T cells in the malignant microenvironment play a role in the pathobiology of FL [3].

The diagnosis is based on histopathology examination of a lymph node biopsy or extranodal affected tissue. FL is B cell neoplasm derived from germinal (follicle) center cells, a mixture of cleaved cells (centrocytes) and non-cleaved large cells (centroblasts).

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Abbreviations

FL	– follicular lymphoma
EZH2	– enhancer of zeste homologue 2
FLIPI	– Follicular Lymphoma International Prognostic Index
PFS	– progression-free survival
OS	– overall survival
PRIMA PI	– PRIMA prognostic index
ISRT	– involved-site radiotherapy 24-30Gy
GELF	– Groupe d`Etude des Lymphomes Follicularies
R	– rituximab
O	– obinutuzumab
CHOP	– Cyclophosphamide, Doxorubicin, Vincristine, Prednisone
CVP	– Cyclophosphamide, Vincristine, Prednisone
B	– bendamustine
POD24	– progression of disease within 24 months
PI3K	– phosphoinositide 3-kinase
BTK	– bruton tyrosine kinase
CAR	– Chimeric antigen receptor

The histological grade of FL depends on the number of centrocytes and centroblasts per high power field according to the current World Health Organization classification [1]. Histological grading is based on counting the absolute number of centroblast per high power field, and characterize FL to grades 1 and 2 (grade 1: 0-5 centroblasts per high power field grade 2: 6-15 centroblasts), which is present in 80% cases. Grade 3 FL has >15 centroblasts per high power field, and is subdivided into FL 3A (still present centrocytes) and FL 3B (composed of centroblasts). Histological grading is significant in the treatment of FL, because grades 1, 2, and 3A should be treated as an indolent disease, but 3B is an aggressive lymphoma and should be treated as diffuse large B-cell lymphoma.

The median age of patients at FL diagnosis is 65 years. Typically, presentation is painless diffuse lymphadenopathy, slowly enlarged over a few weeks or months. B symptoms (unexplained fever > 38°C, night sweats and weight loss > 10% within six months) are present in approximately 20% of patients, but bone marrow involvement is very common in 70% of patients. Staging is performed according to the Ann-Arbor classification. The disease course is characterized by remissions and multiple relapses over time.

Risk assessment is performed by using prognostic indices. Follicular Lymphoma International Prognostic Index (FLIPI) was designed in the pre-rituximab (anti CD20 monoclonal antibody) treatment, but is widely used in the immunochemotherapy era [4]. FLIPI 2 was developed in patients who received rituximab and is predictive of PFS (progression-free survival) and OS (overall survival). PRIMA prognostic index (PRIMA-PI) was developed in FL patients treated with immunochemotherapy followed by rituximab maintenance [5] **(Table 1)**. Although these indices and risk scores are important, they are not able to identify the highest-risk patients who will relapse early within two years of frontline treatment. Recently, a new prognostic score was described as m7-FLIPI, which incorporates clinical characteristics of FL mutations (mutational status of 7 genes). However, it is not standardized and used in daily clinical practice and remains part of the research approach.

Several parameters should be evaluated when making decisions for treating patients with FL: clinical need to start therapy, prognostic parameters, age, comorbidities, performance status, and quality of life. First line treatment in early stage of FL (I/II clinical stage), which is present in less than 20% of FL patients, is involved-site radiotherapy 24-30Gy (ISRT). This therapy can be combined with rituximab monotherapy. Most FL patients have advanced III-IV stage of disease, which is not curable, but a group of these patients are asymptomatic or even show spontaneous regression. Indication to start therapy is high tumor burden based on the GELF criteria (Groupe d`Etude des Lymphomes Follicularies) **(Table 2)**.

Table 1. Adverse prognostic factors according to FLIPI 1, FLIPI 2 and PRIMA-PI
Tabela 1. *Loši prognostički faktori prema FLIPI 1, FLIPI 2, PRIMA-PI*

FLIPI 1	FLIPI 2	PRIMA PI
Age ≥ 60 years/*Starosna dob ≥ 60 godina*	Age ≥ 60 years./*Starosna dob ≥ 60 godina*	/
>4 nodal sites involved *>4 zahvaćenih regiona LČ*	Long diameter of largest LN > 6 cm *Prečnik limfnog čvora > 6 cm*	/
Elevated LDH/*Povišen LDH*	Elevated β_2 M/*Povišen β_2 M*	Elevated β_2 M/*Povišen β_2 M*
Ann Arbor stage III – IV *Klinički stadijum III - IV*	Bone marrow involvement *Infiltracija koštane srži*	Bone marrow involvement *Infiltracija koštane srži*
Hemoglobin < 120 g/l *Hemoglobin < 120 g/l*	Hemoglobin < 120 g/l *Hemoglobin < 120 g/l*	/

FLIPI (Follicular Lymphoma International Prognostic Index): low risk 0-1 risk factor, intermediate risk 2 risk factors, high risk ≥3 risk factors

FLIPI (*Folikularni Limfom Internacionalni Prognostički Indeks): nizak rizik 0-1 faktor rizika, srednji rizik 2 faktora rizika, visok rizik ≥3 faktora rizika*

PRIMA-PI (PRIMA prognostic index): low risk: normal β_2 microglobulin (β_2 M) and bone marrow not involved; intermediate risk: normal β_2 M and bone marrow involved; high risk: elevated β_2 M and bone marrow involved LDH-lactate dehydrogenase, LN-lymph node

PRIMA-PI (*PRIMA prognostički indeks): nizak rizik: normalan β_2 mikroglobulin (β_2 M) bez infiltracije koštane srži; srednji rizik: normalan β_2 M i infiltracija koštane srži; visoki rizik: povišen β_2 M i infiltracija koštane srži LDH-laktat dehidrogenaza, LČ-limfni čvor*

Table 2. GELF criteria for initiation of treatment in patients with follicular lymphoma
Tabela 2. *GELF kriterijumi za započinjanje lečenja bolesnika sa folikularnim limfomom*

Presence of B symptom/*Prisustvo* B *simptoma*
Bulky disease (tumor mass ≥ 7 cm)/*Velika tumorska masa (≥ 7 cm)*
Involvement of ≥ 3 nodal sites, each with a diameter ≥ 3 cm *Zahvaćenost više od 3 limfne žlezde veličine ≥ 3 cm u različitim regionima*
Symptomatic splenomegaly/*Simptomatska splenomegalija*
Organ compression/*Kompresija vitalnih organa tumorom*
Pleural effusion or ascites/*Pleuralni izliv ili ascit*
Elevated lactate dehydrogenase or elevated β2 microglobulin, cytopenia or leukemic phase *Povišena laktat dehidrogenaza ili povišen β2 mikroglobulin, citopenije ili leukemijska faza*

Patients with a low tumor burden, asymptomatic disease, and who do not meet the GELF criteria can be managed by a *"watch and wait"* approach. In asymptomatic, advanced stage FL, rituximab monotherapy may be considered [6]. Immunochemotherapy is standard first-line treatment in patients with symptomatic advanced stages III and IV of the disease. Anti CD20 monoclonal antibody, rituximab (R) or obinutuzumab (O), in combination with chemotherapy such as CHOP (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone), CVP (Cyclophosphamide, Vincristine, Prednisone) or Bendamustine (B) should be used. CVP protocol is inferior to CHOP and Bendamustine in PFS (progression-free survival) rate but similar in OS (overall survival). R-CVP is more suitable for elderly patients or a combination of rituximab with chlorambucil. BRIGHT trial favors R-B immunochemotherapy over R-CHOP because of a lower rate of toxic side effects [7]. In the GALLIUM study, obinutuzumab-chemotherapy was compared to rituximab-based chemotherapy, and a longer PFS was observed with obinutuzumab compared to rituximab (80% vs. 73,3%), although no statistical difference was seen in overall survival and complete remission rates [8]. Obinutuzumab has greater antibody-dependent cellular cytotoxicity than rituximab but has more toxicities, infusion-related reactions and severe infections. Obinutuzumab is indicated for rituximab refractory FL and in patients with advanced stage symptomatic FL with FLIPI score ≥ 2. Patients who respond to immunochemotherapy (partial or complete response) should receive maintenance therapy with rituximab or obinutuzumab every two months for a period of two years [6].

Majority of FL patients respond well to front-line immunochemotherapy, but the disease course is characterized by subsequent relapses and most patients need multiple lines of treatment. At suspected disease relapse or progression, it is very important to perform a new biopsy in order to exclude transformation to aggressive lymphoma. One of the main prognostic indicators for relapsed patients is time to disease progression. Patients experiencing progression of disease within 24 months after initiation of therapy (POD24) have poor outcomes, five year survival of only 50% [9]. Treatment strategies for the management of patients with POD24 are not well established.

There are several therapy options for relapsed/refractory follicular lymphoma. Initiation of treatment is based on GELF criteria and asymptomatic patients with low tumor burden can be observed without therapy. In relapsed patients, it is very important to make a difference between early and late relapse. Early relapse (POD24) should be treated with non-cross resistant immunochemotherapy such as O-Bendamustin or O-CHOP, followed by obinutuzumab maintenance. High-dose therapy followed by autologous stem cell transplantation is an option for patients under 65 years. Allogeneic stem cell transplantation can be considered in selected younger patients with relapse after autologous stem cell transplantation or later high-risk relapses [6]. In patients with early progression after initial therapy, enrollment in clinical trials should always be considered.

Patients who relapsed late after induction immunochemotherapy may be retreated with the same immunochemotherapy regimen, in accordance with the principles of cumulative drug toxicity. In symptomatic patients with low tumor burden, rituximab monotherapy may be applied. Lenalidomide (Revlimid) given with rituximab (so-called R^2) is used as a second-line therapy, and this regimen achieves a median PFS of approximately three years in patients who are not refractory to rituximab. This immunomodulatory, non-chemotherapy strategy was investigated in trials favoring the use of R^2 in older patients with comorbidities. Lenalidomide and rituximab combination showed good results both in first-line treatment, in patients with early and late relapses and refractory FL [10].

Immunochemotherapy has been the standard in FL treatment for a long time, but in the last decade, novel targeted, and cellular therapies and innovative drugs have been identified and hopefully will lead to better outcomes in patients with FL. Phosphoinositide 3-kinase (PI3K) inhibitors represent novel targeted therapy for treating POD24 patients. PI3K inhibitors (idelalisib, duvelisib, umbralisib and copanlisib) are effective but exhibit side effects and require prolonged administration until disease progression [11]. Idelalisib is registered in double refractory FL [6]. Combination therapies, PI3K inhibitors with lenalidomide and rituximab may offer the better efficacy of PI3K inhibitors. Orally bruton tyrosine

kinase (BTK) inhibitor ibrutinib and bcl-2 inhibitor venetoclax have modest activity in relapsed FL.

Bispecific antibodies are a promising treatment for patients with FL. Mosunetuzumab CD20/CD3 bispecific antibody redirects T-cells to target and eliminates malignant B lymphoma cells [12]. Chimeric antigen receptor (CAR) T-cell therapy have encouraging results in relapsed FL, based on result of the ZUMA-5 study [13], but more data will be necessary to define the value of these treatments.

Around 80% of FL have epigenetic mutations and different classes of epigenetic modifiers can serve as potential therapies in FL. EZH2 is a histone lysine methyltransferase gene that is mutated in 20% FL. Tazemetostat, oral inhibitor of EZH2, is currently under investigation in the phase 2 study in relapsed FL and may be a successful epigenetic therapeutic option [14].

In the future, identifying new biomarkers and therapeutic targets will enable personalized approaches to managing each FL patient.

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FROM INBORN ERRORS OF IMMUNITY TO LYMPHOMA – A HEMATOLOGIST'S POINT OF VIEW

OD URODJENIH GREŠAKA IMUNITETA DO LIMFOMA – PERSPEKTIVA HEMATOLOGA

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Summary
After infections, malignancies, lymphomas especially, are the second most frequent cause of death in patients with inborn errors of immunity. Factors predetermining the appearance and aggressiveness of lymphomas include gene defects, defects of immune surveillance and regulation as well as infections with oncogenic viruses. Aggressive non-Hodgkin lymphomas, mostly diffuse large B-cell and Bukit subtypes are predominant in deoxyribonucleic acid repair defects, while Hodgkin lymphoma becomes equally present in patients with defects of immune regulation. Marginal zone and mucosa-associated lymphoid tissue lymphomas, appear to be frequent in defects of antibody production, especially in patients with common variable immune deficiency. The prevalence of Epstein-Barr virus may vary within entities, but there is no entity without at least a few cases of lymphoma and Epstein-Barr virus co-infection. Standard treatment of lymphomas associated with deoxyribonucleic acid repair defects and severe combined deficiencies, is stem cell transplantation. Lymphomas in inborn errors of immunity with a less severe clinical presentation, should be treated with immunochemotherapy and monoclonal antibodies (Brentuximab, Rituximab) wherever feasible. There is no data about the usefulness of checkpoint inhibitors, bi-specific antibodies and T-cells with chimeric antigen receptor. Allogeneic stem cell transplantation represents a major indication for treatment of relapse/refractory lymphomas in any inborn error of immunity. Potential benefit of therapy with Chimeric antigen receptor Natural-killer cells in lymphomas associated with inborn errors of immunity, remains to be seen in future studies.
Key words: Immune System Diseases; Metabolism, Inborn Errors; Lymphoma; Hodgkin Disease; Lymphoma, Non-Hodgkin; Therapeutics

Sažetak
Posle infekcija, maligniteti a naročito limfomi su na drugom mestu kao uzroci smrti pacijenata sa urođenim poremećajima imuniteta. Faktori koji omogućavaju pojavu i agresivnost limfoma su gentski defekti, defekti imunološkog nadzora i regulacije, kao i infekcije onkogenim virusima. Agresivni, ne-Hodgčkinovi limfomi, pretežno difuzni krupnoćelijski B i Burkitov limfom, predominatni su u defektima reparacije dezoksiribonukleinske kiseline, dok je kod bolesnika sa defektom imunske regulacije, učestalost Hodgčkinovog limfoma podjednaka sa prethodnim podtipovima. Marginalno zonalni i limfoidno tkivo povezano sa sluzokožom postaju češći u defektima produkcije antitela, posebno u uobičajenoj varijabilnoj imunodeficijenciji. Prevalencija Epštajn-Barovog virusa varira, ali nema nijednog entiteta u kome bar neki od limfoma nije udružen sa ovom infekcijom. Standardna terapija limfoma udruženih sa defektima reparacije dezoksiribonukleinske kiseline i teškom kombinovanom imunodeficijencijom je transplantacija matične ćelije hematopoeze. Limfome nastale u okviru urođenih grešaka imuniteta sa blažom kliničkom slikom, treba lečiti imunohemoterapijom uz primenu monoklonskih antitela (*Rituksimab, Bretuximab vedotin*) kad god je to moguće. Nema podataka o efikasnosti inhibitora kontrolnih tačaka, bispecifičnih antitela i T-limfocita sa himernim antigenim receptorom kod ovih pacijenata. Alogena transplantacija matičnih ćelija hematopoeze je značajna idnikacija za lečenje relapsirajućih i/ili refraktornih formi limfoma nastalih u bilo kom entitetu sa deficitom imuniteta. Rezultati budućih studija će pokazati potencijalnu korist od terapije ćelijama prirodnim ubicama sa himernim antigenim receptorom.
Ključne reči: bolesti imunog sistema; metaboličke urođene greške; limfom; Hočkinova bolest; non-Hočkinov limfom; terapija

Introduction

There are over 430 acknowledged single gene lesions associated with numerous inborn errors of immunity (IEI), with various clinical presentations, prognosis and complications [1]. Development of malignancy is a common event in many of these entities, with hematological malignancies amounting to 85% of all cancers. Among them, two/thirds are non-Hodgkin lymphomas [2]. Tumorigenesis in IEI is a conse-

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Abbreviations

ADA	– Adenosine deaminase
AIDS	– Acquired Immune deficiency syndrome
ALPS	– Autoimmune lymphoproliferative syndrome
APDS	– Activated PI3 kinase-δ syndrome
APO1	– Apoptosis antigen 1
ATM	– Ataxia Telangiectasia Molecule
CAR-NK cells	– Chimeric Antigen Receptor Natural Killer Cells
CAR-T cells	– Chimeric Antigen Receptor T cells
CMV	– Cytomegalovirus
CTLA4	– Cytotoxic T-lymphocyte associated protein 4
CVID	– Common Variable Immunodeficiency
DLBCL	– Diffuse large B-cell lymphoma
DNA	– Deoxyribonucleic acid
DOCK8	– Dedicator of Cytokinesis 8
EBV	– Epstein Barr Virus
FAS	– apoptosis stimulating fragment
FL	– Follicular lymphoma
G-CSF	– Granulocyte Colony Stimulating Factor
GVHD	– Graft Versus Host Disease
HHV	– Human herpes virus
HLA	– Human Leukocyte Antigen
HSCT	– hematopoietic stem cell transplantation
ICOS	– Inducible T-cell Costimulator
IEI	– inborn errors of Immunity
ITK -IL-2	– Inducible T-cell kinase
IUIS	– International Union of Immunological Societies
NCCN	– National Comprehensive Cancer Network
NFκB1	– Nuclear Factor Kappa Light-chain Enhancer of Activated B cells
PD-1	– Programed cell Death protein 1
PET	– Positron Emission Tomography
PIK3CD	– Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta
PIK3R1	– Phosphoinositide-3-kinase regulatory subunit 1
TNFRSF6	– Tumor necrosis factor receptor superfamily member 6 (Known as FasR)

quence of the complex interplay of many factors. The type of gene defect, combined with defects of immune surveillance and regulation, may predetermine the aggressiveness of lymphoid malignancy. Infection with oncogenic viruses such as, Epstein Barr Virus (EBV), Human herpes virus (HHV) or Cytomegalovirus (CMV), additionally contribute to biology and clinical behavior of lymphomas in IEI setting.

In spite of considerable breakthroughs in understanding and diagnostics of IEI, there are still unsolved issues about management of lymphomas in this susceptible population. The aim of this review is to give a critical view on the characteristics of lymphomas according to type of defects in IEI, and to give an update on current and emerging treatment options.

Characteristics of lymphomas according to IEI entity

The systematic review of Riaz and colleagues has elegantly shown a strong association between IEI entities and subtypes of lymphomas. In deoxyribonucleic acid (DNA) repair defects, the majority of lymphomas were aggressive and originated from

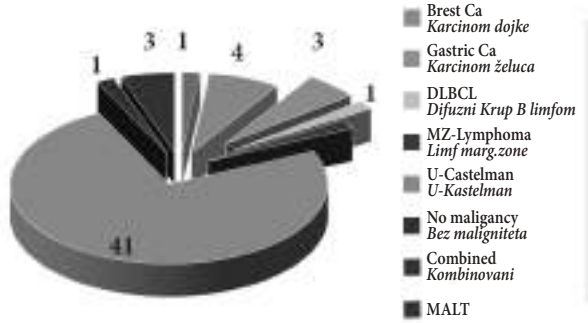

Figure 1. Number malignancies in adult CVID patients from joined cohort from University Clinic Centre Serbia-Belgrade and University Clinical Centre Nis (January 1st 2011 - August 15th 2021)

Slika 1. *Broj odraslih pacijenata sa malignitetom i CVID u objedinjenoj kohorti bolesnika Univerzitetskog kliničkog centra Srbije-Beograd i Univerzitetskog kliničkog centra u Nišu (od 1. januara 2011 do 15. avgusta 2021. godine)*

germinative center B-cells. The highest number of reported cases were patients with Ataxia Telangiectasia and Nijmegen Breakage Syndrome, which had mainly developed Diffuse large B-cell lymphoma (DLBLC) and less frequently Burkitt lymphoma [3]. Both lymphoma types originate from B-cells undergoing affinity maturation in the germinative centers of the lymph nodes. High incidence of DLBCL is therefore highly expected in defective DNA repair setting, since DNA breakage and repair are common events during isotype switching and affinity maturation. It is noteworthy that chronic antigenic stimulation and infection with EBV, contribute to dysregulated B and T-cell proliferation in this patient subgroup.

The incidence and biology of lymphoma subtypes differ in patients with immune regulation defects such as autoimmune lymphoproliferative syndrome (ALPS) and activated PI3 kinase-δ syndrome (APDS). Defects in FAS mediated (TNFRSF6, CD95, and APO1) and other apoptotic pathways in ALPS, lead to lymphoproliferation with a 14-fold risk for non-Hodgkin lymphoma and a 53-fold risk of Hodgkin lymphoma development [4]. This comes as no surprise, since Hodgkin Reed-Sternberg cells have features of activated B-cells, engaged in complex interaction with surrounding reactive tumor microenvironment.

In APDS, gain or loss of function of subunits with regulatory role within PI3 kinase δ pathway, have deleterious effects on T- and B-cell functions. T-cells in APDS are susceptible to apoptosis and cellular senescence, while B-cells could not undergo isotype switching and poorly respond to follicular T-helper cells stimuli [5]. Further lymphoproliferation is driven by EBV infection, whose presence is detected in 30% of APDS patients, of whom 20% develop B-cell lymphoma [6]. For that reason, apart from highly prevalent Hodgkin lymphoma and DLBCL, landscape of lymphoma entities is enriched with MALT and marginal zone B-cell lymphomas.

[7]. The later subtypes develop from primed B-cells residing outside the lymph nodes.

Another disease classified as an immune regulation defect, is X-linked lymphoproliferative disease type XLP1. The lymphoma most commonly diagnosed in this entity is DLBCL, with EBV present in 50% of cases. Straightforward association of EBV with hemophagocytic lymphohistiocytosis and lymphomas has placed this disease among defects with high susceptibility to EBV in latest IUIS 2019 classification [1].

Finally, a group of diseases known as Tregopathies, due to defects of CTLA-4 check point molecule or its accompanying molecules, evolve into lymphomas with frequent EBV positivity [8].

Within the defects of antibody production, Common variable immunodeficiency (CVID) is the most common symptomatic IEI in adults. Adult patients with CVID have 30-fold higher tendency to develop lymphomas, and usually appear in CVID lasting more than 14 years, with average diagnosis in 4^{th} -6^{th} decade [9]. In lymphomas diagnosed in CVID, EBV infection was associated in 31% of cases [2, 9].

When looking at lymphoma entities found in CVID, there is a shift towards Marginal zone and MALT lymphomas, making these subtypes equally or even more frequent than DLBCL and Hodgkin lymphoma. Apart from reports from western national registries, this tendency has been observed in some parts of the Balkans, in Northern Macedonia (unpublished data). Likewise, similar distribution of lymphoma subtypes was found in our joint cohort with 55 patients from two University Clinical Centers Serbia (Belgrade) and Nis, during a 10-year follow up. Of them, 3 patients (5.45%) had DLBCL, another 3 (5.45%) had MALT lymphoma and 1 (1.81%) had Marginal zone B-cell lymphoma **(Figure 1)**. In accordance to data from other studies, our cohort showed a striking absence of follicular lymphomas (FL) in CVID patients [9]. Its noteworthy mentioning that FL holds the second place in overall lymphoma incidence in human population in the western hemisphere, right behind DLBCL. This discrepancy of FL incidence in CVID versus normal population, is probably due to defective signaling (ICOS, PIK3CD, NFKB-1 or PIK3R1 mutations) within germinative centers in CVID patients. The lack of sufficient signals abort the formation of germinative center reaction, which is a prerequisite for the development of FL. It has been hypothesized that malignant B-cells need several reentries into the follicular center, in order to "highjack it" and establish a malignant lymphoma [10, 11].

Therapy of lymphomas in IEI patients

General rules

A literature search for treatment of lymphomas associated with IEI, gave limited data containing mostly case reports and case series. Therefore, the decision on treatment is based on individual judgment and experience of the hematologist or pediatrician.

In general, treatment options do not differ significantly from therapy of immunocompetent patients.

Selection of treatment depends on lymphoma subtype, stage of the disease, patient's age, and comorbidity status. Staging procedure is therefore critical and should be identical to management of non-IEI lymphoma cases, except for patients with DNA repair defects. It is generally acknowledged that diagnostics using X-rays or PET scan as well as radiotherapy, should be strictly avoided in this radio-sensitive subgroup.

Response and overall survival to standard chemotherapy, was inferior in IEI patients as compared to patients without immunodeficiency. Dismal outcomes were due to poorer tolerability of chemotherapy and susceptibility to infectious agents [12]. Besides the need for vigorous treatment of infections, one may advocate the use of antimicrobial prophylaxis. In the absence of clear data, some preventive strategies could be taken from NCCN clinical practice guidelines for AIDS related lymphomas. According to recommendations, granulocyte colony-stimulating factors (G-CSF) and quinolones should be used until resolution of neutropenia, and trimethoprim sulfamethoxazole should be given for pneumocystis jirovecci prophylaxis.

High toxicity rate of epipodophyllotoxins (Etoposide) and alkylating agents (Cyclophosphamide) in patients with DNA repair defects requires its avoidance or dose modifications [13].

Monoclonal antibodies in treatment of lymphomas associated in IEI

Various combinations of chemotherapy with anti-CD20 monoclonal antibody have been gradually introduced, both for pediatric and adult IEI patients with DLBCL [3, 14]. In non IEI patients with DLBCL, FL or chronic lymphocytic leukemia, a type II glycoengineered anti-CD20 monoclonal antibody obinutuzumab has been adapted for standard treatment. Currently there is no data about the use of obinutuzumab in lymphomas associated with IEI. Preclinical studies showed that its effectiveness relies on antibody dependent cell cytotoxicity mediated by pool of NK lymphocytes. This may raise a concern about efficacy of obinutuzumab in IEI with depleted or deficient NK cell function.

Recent study of Pincez and coworkers has brought into focus the beneficial effects of immunoconjugates for selected lymphoma subtypes derived in IEI. Authors have used Brentuximab vedotin, an anti-CD30 monoclonal antibody coupled with microtubule disrupter -monomethyl auristatin E, in 7 IEI patients, of which 6 were carrying DOCK8, ATM, ITK, CD27, ADA, SH2D1A genetic lesions. The drug was given as first line in two anaplastic large cell lymphomas, one DLBCL, one prolymphocytic leukemia case and in one case with mucocutaneous ulcer. Brentuximab was given as second line treatment in 2 patients with relapse/refractory Hodgkin lymphoma. Malignant cells in all cases expressed CD30 molecule, but it varied from 30-100%. This approach induced response

and/or remission in 5 patients, bridging towards hematopoietic stem cell transplantation (HSCT) [15]. This was not the only proof of brentuximab efficacy, since two cases of successful treatment of Hodgkin lymphoma, in patients with CVID and Ataxia telangiectasia, were reported earlier [16, 17].

A recent breakthrough in treatment of lymphomas has been achieved with the introduction of bispecific antibodies. Bispecific antibodies engage domains on the surface of tumor cells with domains of unspecific T-cells, putting them into close proximity. Net result is an activation of T-cells and stimulation of tumor destruction. Unfortunately, certain IEI entities frequently lack functional T-cells, making this approach of limited use in these subtypes. Until now, there is no data about their efficacy in IEI patients with lymphomas.

Another established group of monoclonal antibodies known as "check point inhibitors", act through blocking the inhibitory signals mediated through PD-1 or CTLA-4 receptors. The physiological role of PD-1 and CTLA-4 molecules is to control potential auto-reactive clones and block autoimmune reactions. Abuse by overexpression of PD-1, on the surface of malignant lymphoma cells, particularly on Reed-Sternberg cells, results in resistance to anti-tumor cellular response and tumor progression. Introduction of checkpoint inhibitors in treatment led to satisfactory response rates even in cases with defective T-cell response, seen both in AIDS related Hodgkin and non-Hodgkin lymphomas [18–20]. Important adverse reaction seen throughout the treatment with checkpoint inhibitors, is the appearance of autoimmune diseases or its exacerbation. So far, there is no data about the use of these drugs in patients with IEI related lymphomas. Nevertheless, numerous IEI entities including immune regulation defects, tregopathies, CVID and even some syndromic IEI, may present with autoimmune phenomena and autoimmune diseases. Application of check-point inhibitors, therefore poses a serious threat of life-threatening exacerbation of autoimmune reactions. This was reported in 5 patients with various IEI including DOCK8, CD40 deficit and CVID whose clinical state worsened after minimal dose of pembrolizumab, received to treat progressive multifocal leukoencephalopathy [21].

Cells with chimeric-antigen receptor in treatment of lymphomas associated in IEI

In 2017 and 2018 FDA approved the use of T-cells with chimeric-antigen-receptor (CAR-T cells) for relapsed/refractory B-cell acute leukemia and lymphomas. These autologous cells with "ex vivo" modified T-cell receptor, were able to induce very high responses in patients resistant/refractory B-cell malignancies [22]. Furthermore, applicability and effectiveness of CAR-T cells, was shown even in case reports with AIDS related relapse/refractory lymphomas [23, 24]. In spite of encouraging results, production of autologous CAR-T cells and their efficacy could be hampered by low number of mobilized T-cells, T-cell exhaustion, immune or age-related T-cell senescence, prior therapies, unrecognized underlying genetic defects and many other patient or procedure related factors [25, 26]. Obviously, the concerns mentioned above invariably apply for the majority of IEI patients. Currently, there is no data concerning the use of CAR-T cells or treatment of lymphomas associated with IEI.

A promising approach that might overcome this problem both in IEI and non-IEI patients with lymphomas is development off-the-shelf, ready to use CAR-NK cells.

CAR–NK cell receptor construct is same as in CAR-T cells. The advantages over CAR-T cells is in the absence of alloreactivity, ability to overcome immune evasion by tumor, as well as the absence of cytokine release syndrome and neurotoxicity caused by cell activity [27]. The proof of effectiveness of this concept is the overall response rate in 8/11 patients (73%) with 7 complete responses in a recent pilot study. Of patients with complete remission, 3 had relapse/refractory chronic lymphocytic leukemia and 4 had relapse/refractory non-Hodgkin lymphomas [28]. Meanwhile, the number of phase I and II clinical trials with patients with hematological malignancies is rapidly growing [27]. Having in mind the rationale behind the use of CAR-NK cells, we might expect that this strategy may be feasible in treatment of hematological malignancies associated with IEI. The following years will provide the clue for this assumption.

Hematopoietic stem cell transplant in treatment of lymphomas associated with IEI

Hematopoietic stem cell transplantation (HSCT) is considered the "standard of care" and major therapeutic option particularly in children with severe combined IEI [29]. The decision to undergo HSCT in adolescents, young adults with milder forms of IEI, has become challenging, due to high transplant related morbidity and mortality on one side, and improved conventional management and extended overall survival. In spite of these changes, the development of malignancy associated with IEI still remains one of the major indications for HSCT [29]. Optimal timing for Hematopoietic stem cell transplantation (HSCT) in IEI associated lymphoid malignancies should be in remission of very good partial response. Decisions about stem cell source, conditioning regimes need to take in consideration age, autoimmune manifestations, organ function damage, past and present infections and many other issues [30]. In general, improvement in HLA typing, adoption of reduced intensity regimens, increased availability of alternative stem cell sources and improved methods of GVHD prophylaxis improved outcomes in HSCT in all IEI patents, especially in adolescents and young adults [30–32].

Conclusion

Improvements in understanding, diagnostic and management of patients with inborn errors of immunity extended overall survival and life expectancy, making the appearance of lymphomas no longer limited to childhood. The burden of such patients is there-

fore growing, creating therapeutic challenges both to pediatricians and hematologists. The spectrum of lymphoma subtypes associated with inborn errors of immunity is wide, frequently depending on the type of underlying inborn error defect. Diagnostics and treatment strategies should acknowledge risks and limitations in sensitive patients with deoxyribonucleic acid repair defects. Hematopoietic stem cell transplantation is the standard of care for patients with severe immune defects and short life expectancy even without malignancy. In those with a milder clinical picture and better survival, transplantation should be the treatment of choice for relapsed/refractory lymphomas. Treatment should rely on chemotherapy with the addition of appropriate monoclonal antibodies (rituximab, brentuximab vedotin) wherever feasible. The applicability and effectiveness of new "ready to use, off the shelf" approaches with Chimeric Antigen Receptor Natural Killer cells remains to be elucidated in near future.

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NON HODGKIN LYMPHOMA
NE-HODGČKINOVI LIMFOMI

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PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA – AN OVERVIEW

PRIMARNI LIMFOM CENTRALNOG NERVNOG SISTEMA – PREGLED

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Summary

Introduction. Primary central nervous system lymphoma is a rare entity mostly presenting with non-GCB diffuse large B-cell lymphoma, being confined to the brain, spinal cord, meninges, and eyes. **Diagnosis.** The diagnosis is frequently established by stereotactic or open the brain biopsy, but in some cases with isolated leptomeningeal involvement, the only way is to identify atypical/monoclonal lymphocytes in cerebrospinal fluid. By work-up, we aim to define the extent of disease in the central nervous system and to exclude systemic involvement. **Treatment.** Treatment is tailored according to the patient's age, fitness, vital organ function, comorbidities, and available therapy. The backbone of induction treatment is high-dose methotrexate, usually within polychemotherapy. Consolidation phase is a matter of debate between two approaches: 1. high dose chemotherapy with autologous stem cell transplantation, which appears to be the preferable option for young fit patients, and 2. whole brain radiotherapy, preserved for transplant-ineligible ones. Whole brain radiotherapy has been raising concerns because of frequent cognitive impairment, which has been significantly diminished by reducing the irradiation dose. Despite a comprehensive treatment approach, many patients relapse, and since the prognosis of relapsed/refractory disease is devastating, there is a sense of urgency for novel treatment strategies. Several targeted agents and immunomodulatory drugs have been investigated in the settings of both relapsed/refractory and initial therapy, but with limited success. Ibrutinib monotherapy can induce durable remissions in the first line, but in relapse/refractory settings, the results are controversial. **Conclusion.** Adequate patient selection and new prospective trials should improve survival and preserve the patient's neurological status.

Key words: Central Nervous System Neoplasms; Lymphoma; Signs and Symptoms; Diagnosis; Therapeutics; Methotrexate; Morphological and Microscopic Findings; Positron Emission Tomography Computed Tomography; Magnetic Resonance Imaging

Sažetak

Uvod. Primarni limfom centralnog nervnog sistema je redak entitet koji se najčešće patohistološki prezentuje kao difuzni B-krupnoćelijski limfom *nonGCB* tipa, a vezuje isključivo za mozak, kičmenu moždinu, moždanice i oči. **Dijagnoza.** Dijagnoza se najčešće postavlja nakon biopsije tumorskog tkiva mozga, međutim, u slučaju izolovanog zahvatanja moždanica, jedini način može biti identifikacija atipičnih/monoklonskih limfocita u likvoru. Propratna dijagnostika ima za cilj da utvrdi raširenost bolesti unutar centralnog nervnog sistema i isključi sistemsku bolest. **Lečenje.** Lečenje se usklađuje sa starošću pacijenta, njegovim opštim stanjem, funkcijom vitalnih organa, komorbiditetima i dostupnom terapijom. Srž indukcione terapije su visoke doze metotreksata obično u okviru polihemioterapije. Faza konsolidacije je tema sporenja između dva pristupa; s jedne strane je visokodozna hemioterapija praćena autolognom transplantacijom matičnih ćelija hematopoeze, što je terapija prvog izbora za mlađe pacijente u dobrom opštem stanju, a sa druge zračna terapija mozga koja je u prednosti kod nepodobnih za transplantaciju. Uloga zračne terapije se preispituje s obzirom na čestu pojavu postiradijacionog kognitivnog slabljenja što je značajno umanjeno primenom redukovanih doza zračenja. Uprkos sveobuhvatnom terapijskom pristupu, kod velikog broja pacijenata dođe do relapsa. Imajući u vidu veoma nepovoljnu prognozu relapsa/refraktorne bolesti, postoji velika potreba za novim terapijskim opcijama. Nekoliko ciljanih lekova i imunomodulatornih agenasa je u fazi istraživanja u terapiji kako relaps/refraktornih, tako i *de novo* pacijenata, međutim, sa ograničenim efektom. Monoterapija ibrutinibom može dovesti do dugotrajnih remisija u prvoj liniji terapije, dok su u lečenju relaps/refraktornih pacijenata rezultati kontroverzni. **Zaključak.** Adekvatan izbor pacijenata i nove prospektivne studije su osnova za dalje poboljšanje rezultata lečenja, uključujući i očuvanje neurološkog statusa pacijenata.

Ključne reči: tumori centralnog nervnog sistema; limfomi; znaci i simptomi; dijagnoza; terapija; metotreksat; morfološki i mikroskopski nalazi; PET CT; MRI

Introduction

Primary central nervous system lymphoma (PCNSL) represents an aggressive type of extranodal

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Abbreviations

GCB	– germinal center B-cell
PCNSL	– primary central nervous system lymphoma
CNS	– central nervous system
OS	– overall survival
DLBCL	– diffuse large B cell lymphoma
GEP	– gene expression profiling 1
ABC	– activated B cell
PIOL	– primary intraocular lymphoma
SEER	– Surveillance, Epidemiology, and End Results
CSF	– cerebrospinal fluid
CE-MRI	– contrast-enhanced magnetic resonance imaging
BBB	– blood-brain barrier
T1W	– T1-weighted
T2W	– T2-weighted
PET/CT	– positron emission tomography/computed tomography
^{18}FDG	– ^{8}F fluorodeoxyglucose
BMB	– bone marrow biopsy
LDH	– lactate dehydrogenase
ECOG PS	– Eastern Cooperative Oncology Group performance status
IELSG	– International Extranodal Lymphoma Study Group
MSKCC	– Memorial Sloan-Kettering Cancer Center
KPS	– Karnofsky performance score
HD-MTX	– high-dose methotrexate
WBRT	– whole brain radiotherapy
HDC	– high-dose chemotherapy
ASCT	– autologous peripheral blood stem cell transplantation
PFS	– progression-free survival
R	– rituximab
A	– cytarabine
NT	– neurotoxicity
MPV, MTX	– vincristine, and procarbazine
rdWBRT	– reduced dose WBRT
CR	– complete response
PR	– partial response
MATRix	– MTX, cytarabine, thiotepa, and rituximab
ORR	– overall response rate
R/R	– relapse/refractory
R-IE	– rituximab, ifosphamide, etoposide
PD-L1	– programmed death ligand
BTKi	– Bruton's tyrosine kinase inhibitor
TEDDi-R	– temozolomide, etoposide, liposomal doxorubicin, dexamethasone, and rituximab
mTOR	– the mammalian target of rapamycin

lymphoma, with only 2–4% of all primary CNS malignancies and 4–6% of all extranodal lymphomas attributed to PCNSL. It affects around 0.5/100000 persons per year with 65 as the median patient age at diagnosis and with a slight predominance of men [1]. PCNSL refers exclusively to the presence of lymphoma in the brain, spinal cord, leptomeninges, and eyes, without systemic disease involvement. Disease localization in the brain is by far the most prevalent, followed by spinal cord involvement at around 15%. At the same time, a few percent of cases primarily engage meninges or eyes and out-of-eye adnexa lymphomas which are not considered PCNSL [2]. Immunosuppression is the only established risk factor for PCNSL development [3]. In spite of improvement in treatment strategies, the prognosis of PCNSL remains poor, with the 5-year overall survival (OS) rate being somewhat above 30% [2].

Histology of PCNSL

Diffuse large B-cell lymphoma (DLBCL) is the most frequent histological type of PCNSL, appearing in approximately 90% of patients [4]. The majority are unexpected of activated B-cell (ABC) pattern according to immunohistochemistry or gene expression profiling. Clinical Advisory Committee recently reported a proposal of the International Consensus Classification of mature lymphoid, histiocytic, and dendritic cell tumors in which the debate was initiated on whether the term "Extranodal Lymphoma ABC (non-germinal center B-cell (GCB)) type" should be used for a group of DLBCL arising in immune-privileged sites. This group would have encompassed primary DLBCL of CNS, primary testicular DLBCL, primary cutaneous DLBCL, leg type, primary breast DLBCL, intravascular large B-cell lymphoma, and primary adrenal lymphoma as they all share an asymmetric predominance of non-GCB/ABC pattern as well as some molecular features characterizing the DLBCL MCD/C5 genetic subgroup [5]. Very rarely, PCNSL may appear as indolent B-cell lymphoma (from highest frequency: marginal zone, follicular, small lymphocytic, mantle cell lymphoma) or peripheral T-cell lymphoma [2].

Clinical presentation

Various symptoms and clinical signs may precede the diagnosis of PCNSL, mainly depending on disease localization. Up to 70% of patients exhibit some form of focal neurologic deficit, followed by cognitive decline and behavioral disorders in 40–50% of patients. Symptoms of increased intracranial pressure such as headache, nausea, and vomitus are present in one-third of patients and can remain unrecognized for a while if appear isolated. Seizures are uncommon but unambiguously lead to the accurate diagnostic pathways [6-8]. Primary intraocular lymphoma (PIOL) implicates the involvement of eye structures without ocular adnexa and CNS, most commonly affecting vitreoretinal space. This subtype often precedes spreading to the CNS and may present with subtle vision disturbances such as visual acuity, blurring, and floaters. However, in a majority of cases, PCNSL itself does not result in compromised vision [9]. PCNSL with constitutional symptoms requires detailed evaluation in order to exclude systemic disease or infectious complications since those appear in exceptional cases [10].

Diagnosis

Histopathological verification of tumor tissue is essential for diagnosis of PCNSL. The specimen for the analysis is preferably obtained by stereotactic brain biopsy, while craniotomy with open brain biopsy and, more often, wide tumor resection, may produce severe and permanent neurological dys-

function. However, the latter may be considered for a subset of patients with accelerated neurological impairment due to brain herniation or ventricle dilatation [3]. Moreover, in a large retrospective study from Surveillance, Epidemiology, and End Results (SEER) Program database, in a population of 3,543 PCNSL patients, tumor resection plus chemotherapy showed an advantage in terms of overall survival over chemotherapy only, gross resection more than subtotal [11]. Cerebrospinal fluid (CSF) sampling can be diagnostic, thus avoiding tumor biopsy and iatrogenic neurological deficit. Nevertheless, only 15% of patients with PCNSL in a systematic review by Morell et al. exhibited positive CSF, while in the same paper, an analysis of single center experience with 28 PCNSL patients revealed negative CSF findings in all of them [12]. CSF should be generally assessed for blood cell count with differential glucose and protein levels, cytology, flow cytometry and, if available, tested for immunoglobulin heavy chain gene rearrangement [10]. CSF analyses are particularly useful in leptomeningeal disease with no available explicit tumor mass convenient for biopsy. Primary vitreoretinal lymphoma, the most common type of PIOL, is diagnosed by vitrectomy or aspiration of the vitreous humor. Corticosteroid treatment should be avoided before tumor biopsy as around 40% of PCNSL cases experience symptom relief and radiographic regression [1, 3]. However, knowing that many patients eventually receive immediate steroid treatment due to clinical deterioration, if possible, steroids should be withheld for at least a week before obtaining a biopsy [3]. Of note, 50-85% of patients can not be confirmed with lymphoma diagnoses under steroid treatment [1].

MRI and PET in work-up of PCNSL

Contrast-enhanced magnetic resonance imaging (CE-MRI) represents the method of choice for verifying the extent of the tumor mass and evaluating treatment response as well as narrowing differential diagnosis, guiding the biopsy, and contributing to risk assessment [1]. In newly diagnosed patients, CE-MRI reveals supratentorial solitary lesions in 60-70%, most of them involving the periventricular region, deep white matter, basal ganglia, and corpus callosum [13]. In 30-40% of patients, the disease presents with multifocal lesions, while infratentorial localization and spinal cord involvement do not seem to be uncommon [14]. Images from T1-weighted (T1W) CE-MRI define the extent of baseline disease and response to treatment on the basis of contrast enhancement intensity. The main limitation of T1W imaging stems from its capability to measure a tumor only at sites where the blood-brain barrier (BBB) has been disrupted, thus underestimating total mass volume as the parts with intact BBB cannot be detected [14]. Moreover, there is evidence that PCNSL is rather a whole brain disease than one or few radiographically seen lesions [15]. However, contrast enhancement is intense and homogenous in most PCNSL cases, while 6 to 17% of patients show inhomogeneous or missing enhancement patterns [1]. T2-weighted (T2W) sequences provide information about tissue water content and can be helpful for defining the magnitude of perilesional edema as well as infiltration compartments without BBB disruption [16, 17]. Differential diagnosis includes glioblastomas, metastases, tumefactive demyelinating lesions, granulomatous and infectious diseases [13]. For a more accurate determination of the nature and extent of CNS lesions by enlightening their biological and physiological features, advanced MRI techniques of diffusion, perfusion and spectroscopy are being used in clinical practice, depending on the expertise of operator [4].

Brain positron emission tomography/computed tomography (PET/CT) with ^{18}F fluorodeoxyglucose (^{18}FDG) as a biomarker has not yet been widely accepted in routine clinical practice mostly because of physiologically increased ^{18}FDG uptake by several CNS areas which interfere with tumor uptake of the biomarker. Even ^{18}FDG brain PET/CT demonstrated relatively high sensitivity and specificity in several studies with PCNSL, new biomarkers on the horizon, such as ^{18}F fludarabine and ^{68}Ga-Pentixafor, a CXCR4-directed tracer, might improve its reliability in this setting [4, 18].

Staging and risk stratification

There is no conventional staging in PCNSL; knowing the disease verified out of CNS excludes the diagnosis of PCNSL, given that only lymphoma confined to CNS defines PCNSL, while all the other localizations imply secondary disease spreading and require a substantially different treatment approach [10]. Whole body ^{18}FDG PET/CT, bone marrow biopsy (BMB)/aspiration, and testicular ultrasound reveal systemic disease in 4-12% of patients with assumed PCNSL [3]. The first aforementioned is a widely adopted tool in workup of PCNSL as it can substitute CT, testicular ultrasound, and BMB, although with variable accuracy in terms of the latter method. However, PET/CT demonstrated high concordance with the results of BMB in aggressive B-cell lymphomas that comprise most of PCNSL [19]. Ocular examination with a slit lamp by an experienced ophthalmologist is necessary for the exclusion of vitreoretinal involvement [3]. Every patient with confirmed PCNSL should undergo lumbar puncture for CSF evaluation which commonly shows increased cellularity and protein level, while glycorrhachia usually remains normal in opposite to decreased CSF glucose in neuroinfection [3]. CSF analysis for diagnostic purposes was discussed above. Complete blood count, biochemistry analyses, including serum lactate dehydrogenase (LDH) level, and serology for hepatitis B and C and HIV are mandatory, as well as echocardiography, pulmonary function tests, neuropsychological evaluation, and mobility assessment using Eastern Cooperative Oncology Group performance status (ECOG PS) [1, 3]. Comprehensively conducted workup is a good ba-

Table 1. Prognostic models for risk-stratification of PCNSL patients
Tabela 1. *Prognostički modeli za stratifikaciju rizika pacijenata sa PCNSL*

Prognostic models variables *Prognostički modeli varijabli*	IELSG[20] (2003) N=378	NB[21] (2004) N=77	MSKCC[22] (2006) N=338	AMC[23] (2017) N=77	Taipei[24] (2019) N=101	LLR[25] (2021) N=248
Age*/*Starost**	+	+	+	+	+	
ECOG PS/KPS**/*ECOG PS/KPS***	+	+	+		+	
Multifocal lesions/deep brain lesions*** *Multifokalne lezije/Lezije dubokih struktura mozga****	+	+		+	+	
high CSF-proteins/*Povišeni protein u likvoru*	+			+		
LDH****/*LDH****	+					+
ALC (x10^9/L)/*ABL (x10^9/L)*						+
Risk category/*Kategorija rizika*						
Low/*Nizak*	0-1	0	1 (≤50y)	0	0	≤166.8
Intermediate/*Intermedijerni*	2-3	1	2 (>50y, KPS≥70)	1	1	
High/*Visok*	4-5	2	3 (>50y, KPS<70)	2	2	>166.8
Very high/*Veoma visok*		3		3	3	

Legend. IELSG International Extranodal Lymphoma Study Group, NB Nottingham-Barcelona, MSKCC Memory Sloan Kettering Cancer Center, AMC Asan Medical Center, LLR LDH to lymphocyte ratio, ECOG PS Eastern Cooperative Oncology Group Performance Status, KPS Karnofsky Performance Status, CSF Cerebrospinal fluid, LDH Lactate dehydrogenase, ALC Absolute lymphocyte count; *Age: in IELSG > 60, in NB ≥ 60, in MSKCC ≥ 50, in AMC ≥ 65 in Taipei ≥ 80, **KPS in MSKCC score, ECOG PS in all the others; ***multifocal lesions in NB and AMC, deep brain lesions in IELSG and Taipei; ****LDH > 1x upper limit normal in IELSG, absolute value (IU/L) in LLR score

Legenda. IELSG Internacionalna studijska grupa za ekstranodalne limfome, NB Notingem-Barselona, MSKCC Memory Sloan Kettering *centar za rak, AMC Mdicinski centar Asan, LLR odnos LDH i limfocita, ECOG PS Performans status Istočne kooperativne onkološke grupe, KPS Karnofski performans status, CSF likvor, LDH Laktat dehidrogenaza, ABL apsolutni broj limfocita; *Starost: kod IELSG > 60, kod NB ≥ 60, kod MSKCC ≥ 50, kod AMC ≥ 65 kod Taipei ≥ 80, **KPS kod MSKCC skora, ECOG PS kod svih ostalih; ***multifokalne lezije kod NB i AMC, lezije dubokih struktura mozga kod IELSG i Taipei; ****LDH > 1x gornje granice normalnog opsega kod IELSG, apsolutna vrednost (IU/L) kod LLR skora*

sis for the appropriate risk stratification and consequently, proper treatment decisions.

In a retrospective study from SEER database on more than 3500 PCNSL patients managed in the United States of America between 1975 and 2017, age, gender, time of diagnosis, and pathological type were found to be independent indicators of survival in multivariate analysis [2]. However, the database lacks a number of prognostically significant features. Several risk stratification models were developed during last two decades for patients with PCNSL, prevalently composed of elementary clinical, radiographic, and/or laboratory characteristics [20–25]. International Extranodal Lymphoma Study Group (IELSG) score is the most broadly used prognostic model in routine practice and clinical trials. It was established based on the results of the retrospective study by Ferreri et al., which defined age > 60 years, ECOG PS > 1, elevated LDH, hyperproteinorrhachia, and involvement of deep brain structures (periventricular regions, basal ganglia, brainstem, and/or cerebellum) to be independently associated with decreased survival rate. Depending on the number of unfavorable features IELSG score divided PCNSL patients in three risk groups: low (0-1), intermediate (2-3), and high (4-5) [20]. Simplification of risk stratification was introduced with Memorial Sloan-Kettering Cancer Center (MSKCC) score which combines only age and Karnofsky performance score (KPS) to stratify patients into three classes: class 1 (age < 50), class 2 (age ≥ 50 and KPS ≥ 70), and class 3 (age ≥ 50 and KPS < 70) [22]. Various prognostic scores for patients with PCNSL described since are shown in **Table 1.**

Treatment considerations

Front-line treatment

There are no firm recommendations for the treatment of PCNSL due to the lack of randomized phase III clinical trials. In general, the therapy of PCNSL is consisted out of the induction and consolidation treatment phase. High-dose methotrexate (HD-MTX) is the backbone of upfront induction treatment followed by consolidation with either whole brain radiotherapy (WBRT) or high-dose chemotherapy (HDC) plus autologous peripheral blood stem cell transplantation (ASCT) [10]. There is a significant gap between results of published trials and real-world experience, which stems from fairly delimitating inclusion criteria that leaves frail, poorly mobile and comorbidities overloaded patients out of clinical trials. Historically, it was shown that HD-MTX combined with other chemotherapy and/or WBRT is more effective than MTX or WBRT alone in terms of both progression-free survival (PFS) and OS [10].

The addition of rituximab to MTX-based polychemotherapy did not bring clear benefits, as there are randomized trials with inconsistent results. In

the IELSG-32 phase 2 trial, rituximab (R) added to MTX and cytarabine (A) resulted in prolonged PFS (7y- PFS 20% in MTX-A vs. 29% in R-MTX-A arm) and OS (7-y OS 26% in MA vs. 37% in RMA arm) [26]. On the other hand, HOVON 105/ALLG NHL 24 phase 3 randomized trial did not show any benefit of combining rituximab with MTX-based polychemotherapy (MTX, carmustine, teniposide, and prednisone) [27]. A meta-analysis has confirmed that there is no evidence that the addition of rituximab to MTX-based polychemotherapy improves OS, although it may prolong PFS, without a significant increment in the treatment toxicity [28].

Many debates are still ongoing about the place of WBRT in PCNSL treatment considering its proven neurotoxicity (NT), mostly in the form of severe cognitive impairment. Gavrilovic et al. showed in a prospective clinical trial investigating the addition of WBRT (45 Gy in total per patient) to MTX, vincristine, and procarbazine (MPV) that 46% of patients that received adjuvant WBRT developed some form of treatment-related NT at a median of 15 months after radiotherapy. Notably, in a subgroup of patients older than 60 years that were irradiated by adjuvant, 75% developed NT without survival benefit. However, it is important to note that this subgroup consisted of 12 patients [29]. Considering mentioned NT, a study group within Memory Sloan Kettering Cancer Center (MSKCC), conducted further trials, with reduced dose WBRT (rdWBRT, 23.4 Gy) in consolidation (for patients achieving complete remission after initial therapy) along with two cycles of cytarabine, after initial R-MPV chemoimmunotherapy, confirming the absence of statistically significant treatment-related NT with clear survival advantage [30–32]. Morris et al. published results of long-term follow-up of a trial examining R-MPV+rdWBRT+A, pointing out a high 3-y OS of 87% among patients (60%) that received rdWBRT after the complete response (CR) in induction treatment [31].

Apart from WBRT as consolidation therapy there is also ASCT following HDC which has been lately established as a preferred option for young fit patients [3]. The results of the second randomization of IELSG-32 trial reveal comparable survival of patients treated either with consolidative WBRT or HDC+ASCT, but at the cost of significant cognitive decline in WBRT arm received [33]. Nonetheless, it should be noted that patients randomized in WBRT armreceived 36 Gy on the whole brain plus a boost of 9 Gy on the tumor bed if a partial response (PR) was previously achieved in the induction phase with MTX, cytarabine, thiotepa, and rituximab (MATRix) [33]. This is significantly more intensive brain irradiation than in trials conducted by a study group from MSKCC (23.4 Gy), and the comparison between these two approaches is difficult. The study group from MSKCC carried out another study examining R-MPV as the induction before ASCT consolidation, exhibiting excellent disease control (2-year PFS and OS were 81% for the whole group) without evidence of NT [34]. Long-term follow-up of randomized phase 2 PRECIS study demonstrated advantage in 8-y event-free survival in ASCT arm over WBRT with 40 Gy (67% and 39%, respectively, $p = 0.03$), but without OS benefit (69% and 65%, respectively). Conversely, a significant deterioration in balances (52% vs. 10%, $P \leq 0.001$) and neurocognition (64% vs. 13%, $P < .001$) was observed in WBRT over ASCT arm, suggesting avoidance of 40 Gy WBRT in consolidation within the first line treatment [35]. A systematic review and meta-analysis of 13 prospective trials encompassing 348 PCNSL patients treated with ASCT revealed a pooled overall response rate (ORR) of 95%,a CR rate of 80%, and a relapse rate of 19%. Also, pooled 2-y PFS and OS were 74% and 80%, respectively, while 5-y PFS and OS were 65% and 69%, respectively. Overall treatment-related mortality was an acceptable 3%. In the same analysis, consolidations with ASCT and WBRT were compared, demonstrating no difference in response, survival, and relapse rates, but preserved or improved cognitive functions after ASCT [36]. For elderly and frail patients, consolidation with ASCT is not an option, but if cardiac and renal function is preserved (left ventricle ejection fraction ≥ 45%, absence of poorly controlled coronary artery disease and arrhythmias), the patients should be treated with HD-MTX based regimens that include alkylating agents (e.g., procarbazine, temozolomide), and rituximab followed by consolidation with rdWBRT or maintenance with alkylating agents [3, 31]. For unfit patients unable to tolerate chemotherapy, palliative WBRT or high dose dexamethasone remain the options, but with the poor disease control [3]. A report of two 80-year-old individuals with PCNSL treated successfully with ibrutinib monotherapy promises the perspective for this group of patients [37]. Main clinical trials for the front-line PCNSL treatment are summarized in **Table 2**. While awaiting the results of studies related to novel agents in upfront PCNSL treatment, combined chemoimmunotherapy with consolidation depending on patient's and disease characteristics remains the best available option in front-line settings.

Treatment of relapsed/refractory disease

Definition of relapse/refractoriness (R/R) of PCNSL, as well as initial baseline evaluation, has been given by the International PCNSL Collaborative Group in the Report of the international workshop from 2005 [38]. After first-line treatment completion, brain MRI is recommended in a follow up every three months for the first two years, then every six months up to 5 post-treatment years, and finally yearly afterward [39]. High rate of disease recurrence characterizes PCNSL despite improved treatment strategies. Most of the relapses occur within the first two years of follow-up, but late relapses, even ten years after diagnosis, have also been described [10]. Up to 2/3 of patients eventually relapse, according to different reports; the longer the follow-up is, the further the progression of the disease [40]. More often, disease relapse occurs in the

Table 2. First-line treatment regimens in PCNSL (adapted from Schaff et al, Blood 2021) (10, 35)
Tabela 2. *Protokoli u prvoj liniji terapije u PCNSL (adaptirano iz Schaff et al, Blood 2021) (10, 35)*

	Regimen *Protocol*	ORR *ORR*	2y-PFS *2g-PFS*	Treatment-related deaths *Smrt povezana sa lečenjem*
DeAngelis 2002	MVP+WBRT+IT M	94%	50%	8%
Morris 2013	R-MVP+Ara-C+low dose WBRT	95%	77%	6%
Ferreri 2009	M+Ara-C (MA)+WBRT	69%	38%	8%
Thiel 2010[71]	M/WBRT vs. M	53% (in all patients)	43.5% vs. 30.7%	5%
Rubenstein 2013	MT-R	66%	57%	2%
Omuro 2015	R-MVP+ASCT	96%	79%	9%
Omuro 2015	MT vs. MVP	71% vs 82%	39% vs. 58%	10% vs. 6%
Ferreri 2016	MA vs MARix vs. MATRix	40%/51%/65%	36%/46%/61%	6%
Bromberg 2019	R-MBVP vs. MBVP	81% vs. 75%	43% vs. 37%	2% vs. 3%
Houillier 2019	R-MBVP+RAraC + WBRT vs. ASCT	76% vs. 64%	63% vs. 87%	2% vs. 11%

Legend. ORR overall response rate (complete response + partial response), PFS progression free survival, AraC cytarabine, IT M intrathecal methotrexate, MVP: methotrexate, vincristine, procarbazine, WBRT whole brain radiotherapy, M methotrexate, MT-R rituximab, methotrexate, temozolomide, R-MVP rituximab, methotrexate, vincristine, procarbazine, ASCT high dose chemotherapy with autologous stem cell transplantation MT methotrexate, temozolomide, MA methotrexate, cytarabine, MARix methotrexate, cytarabine, rituximab, MATRix methotrexate, cytarabine, thiotepa, rituximab, R-MBVP rituximab, methotrexate, carmustine, teniposide, prednisone, MBVP methotrexate, carmustine, teniposide, prednisone

Legenda. ORR stopa ukupnog odgovora (kompletni odgovor + parcijalni odgovor), PFS preživljavane bez progresije, AraC citarabin, IT M intratekalni metotreksat, MVP: metotreksat, vinkristin, prokarbazin, WBRT radioterapija celog mozga, M metotreksat, MT-R rituksimab, metotreksat, temozolomid, R-MVP rituksimab, metotreksat, vinkristin, prokarbazin, ASCT visokodozna hemioterapija sa autolognom transplantacijom matičnih ćelija hematopoeze, MT metotreksat, temozolomid, MA metotreksat, citarabin, MARix metotreksat, citarabin, rituksimab, MATRix metotreksat, citarabin, tiotepa, rituksimab, R-MBVP rituksimab, metotreksat, karmustin, tenipozid, prednizon, MBVP metotreksat, karmustin, tenipozid, prednizon

elderly and frail, primarily because of the initial suboptimal treatment approach. In a large cohort of PCNSL patients (n=563) treated mostly with HD-MTX-based therapy (92.6%), nearly 30% of patients were primary refractory, while after a median of nine months of follow up 16.5% relapsed. Among these, 45.5% R/R median PFS was 2.2 months, and OS 3.5% [41]. R/R PCNSL thus represents genuinely an unmet need with no established treatment approach. Therefore, entering a clinical trial is the first treatment option for these patients [3, 39]. If there is no such opportunity, treatment decision should be based on patient fitness, previous therapy, and duration of response after the first line. For patients exhibiting response after HD-MTX-based treatment longer than 24 months, retreatment with HD-MTX appears to be the reasonable and acceptable approach resulting in high ORR rates (up to 90%) [3]. In a retrospective study of 39 patients receiving MTX-based retreatment, median PFS was 16 months, 1-year OS was 79% (95%CI 63–89), and median OS was 41 months [42]. On the other hand, refractory and/or earlier relapsed patients should undergo high dose iphosphamide containing protocols, particularly rituximab, iphosphamide,and etoposide (R-IE) ,which results in ORR of 38% and 2-y OS after relapse of twenty five months [3]. Further, consolidative therapy with either HDC with ASCT or WBRT is recommended, depending on previously delivered treatment, patient's fitness and comorbidities, and availability of required treatment options [3]. HDC followed by ASCT showed median PFS of 11.6 months and 2-y OS of 45% in the R/R setting [43]. The usefulness of agents like pemetrexed, temozolomide, topotecan, and rituximab appeared to be limited showing ORR between 31–55% and PFS of 1.6-5.7 months [44].

A number of clinical trials, including novel agents, have been conducted in an attempt to overcome the devastating prognosis of R/R PCNSL. Due to the findings demonstrating over-expression of programmed death ligand 1 (PD-L1) and its corresponding cell surface protein PD1 on the biopted tissue of PCNSL and evidence of a presence of mechanisms of PD-1 T-cell mediated immune evasion, immune check-point inhibitors have been investigated in monotherapy as well as in combination with other agents in the treatment of R/R PCNSL [10]. Based on the positive results of a few retrospective series,the PD-1 inhibitor, nivolumab, has been prospectively investigated in R/R PCNSL as monotherapy in R/R PCNSL and primary testicular lymphoma. However results are not yet available (NCT02857426). Also, several clinical trials are ongoing, testing nivolumab plus ibrutinib (NCT03770416) and nivolumab plus pomalidomide (NCT03798314). In addition, nivolumab has been investigated in upfront setting as maintenance therapy (NCT04022980 and NCT04401774), as well as a part of initial treatment with rituximab, MTX, and lenalidomide (NCT04609046) [45]. The results of these studies are yet to be public. Immunomodulatory drugs, such as lenalidomide and pomalidomide, have also exhibited

effectiveness in R/R PCNSL with ORRs after lenalidomide and pomalidomide alone being 62% and 43%, respectively, while the combination of lenalidomide and rituximab resulted in ORR 63% [10]. Numerous trials with lenalidomide upfront (NCT04609046, NCT04446962, NCT04481815, NCT04737889) and maintenance (NCT04120350, NCT03495960, NCT04627753) and few in R/R setting (NCT03703167, NCT04129710) are still ongoing [45]. Probably the most promising, widely investigated agent in the treatment of PCNSL is first-in-class Bruton's tyrosine kinase inhibitor (BTKi), ibrutinib. Initially investigated in the R/R PCNSL, ibrutinib monotherapy yielded satisfactory ORRs of 52% with a daily dose of 560mg (n=52, phase 2), and 77% with 840 mg daily (n=13, phase 1), while in combination with MTX and rituximab (n=15, phase 1) ORR was 80%. However, no sustainable PFS was observed after the initial response (4.8 months, 4.6 months, and 9.2 months, respectively, in monotherapy with 560 mg, 840 mg, and combination setting) [45]. Another phase 1 clinical trial with ibrutinib in combination with subsequent administration of temozolomide, etoposide, liposomal doxorubicin, dexamethasone, and rituximab (TEDDi-R) (n=18, 5 initial + 13 R/R PCNSL) showed initial radiographic regression of tumor mass in 94% of patients after first two weeks of ibrutinib monotherapy, prior to administration of the rest of the protocol. Complete remission after TEDDi-R was achieved in 86% of evaluable patients, while 67% remained disease-free after two years of follow-up. Nevertheless, frequently severe and even fatal pulmonary or cerebral aspergillosis raised the question about the eligibility of patients receiving corticosteroids or being previously heavily pretreated to safely use ibrutinib in this setting [46]. Second generation BTKi, tirabrutinib, has also exhibited efficacy comparable with ibrutinib, without durable disease control as well [47]. BTK inhibitors are currently investigating several clinical trials in both upfront and R/R settings [45]. BTK inhibitors have been investigated currently in several clinical trials in both upfront and R/R settings [45]. One of the first targeted agents examined in R/R PCNSL patients, the mammalian target of rapamycin (mTOR) inhibitor, tacrolimus, exhibited relatively satisfactory ORR. However, very short PFS and significant treatment-associated mortality, thus losing perspective for further investigation in this setting [48].

Interpretation of the results of most of the trials mentioned is significantly compromised by small numbers of participants, mixed de novo and R/R patients or both primary and secondary CNS lymphoma in the same trial, as well as inconsistent previous treatment, which all may be the reflection of disease rarity. Notwithstanding the insufficient disease control with targeted and immunomodulatory agents proven so far, several clinical trials investigating their efficacy and safety in monotherapy or in a palette of combinations are ongoing, and many questions are yet to be answered with the pending results.

Conclusion

Primary central nervous system lymphoma represents a rare entity with a dismal prognosis despite significant advances in treatment over the years. Reasons at the core for bleak prognosis include unfavorable histology in the majority of patients, specificities of blood-brain barrier, and numerous unfit patients ineligible for aggressive treatment. The latter is closely associated with the disproportion of survival results in prospective clinical trials as opposed to everyday clinical practice. Workup may consist of whole-body computed tomography, bone marrow biopsy, testicular ultrasound, slit lamp ocular examination, analysis of cerebrospinal fluid, and standard laboratory and functional examination. Initial treatment is multimodal, consisting of induction with procarbazine-based therapy, followed by consolidation with either high-dose chemotherapy plus autologous peripheral blood stem cell transplantation or whole brain radiotherapy switching to reduced doses in order to avoid neurotoxicity. However, many patients eventually relapse with subsequent short survival. Clinical trial is the therapy of choice for relapse/refractory primary central nervous system lymphoma, reflecting unmet clinical needs in this setting. Novel agents are emerging within the early phases of clinical trials, both in monotherapy and as a part of different drug combinations. Ibrutinib is the most investigated novel agent in the treatment of primary central nervous system lymphoma with promising results, but it bears a potentially high toxicity burden due to frequent severe aspergillosis. Many clinical trials are ongoing with the hope of better disease control in different patient subgroups, including unfit and frail.

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RISK-STRATIFICATION IN DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA

STRATIFIKACIJA RIZIKA KOD BOLESNIKA SA DIFUZNIM B-KRUPNOĆELIJSKIM LIMFOMOM U ERI PRIMENE RITUKSIMABA

Olivera MARKOVIĆ

Summary

Introduction. Diffuse large B-cell lymphoma represents a group of entities characterized by pathological and biological heterogeneity and different clinical outcomes. Due to pronounced heterogeneity, prognostic biomarkers are of great importance in identifying high-risk patients who might benefit from more aggressive approaches or new therapeutic modalities. Several prognostic score systems have been established and applied to predict the survival of patients with diffuse B-large cell lymphoma. The first established prognostic system for NHL patients is the International Prognostic Index, its variations Revised International Prognostic Index and National Comprehensive Cancer Network-International Prognostic Index were subsequently introduced in the era of immunochemotherapy. As the discriminative power of clinical scores is suboptimal, other strategies have been explored in order to improve risk stratification, especially in the high-risk group of patients who have the highest risk of treatment failure. In this regard, there is a tendency to integrate genetic and molecular biomarkers and prognostic somatic mutations into standardized and personalized models for risk stratification that would have a wide application in routine clinical practice. The results of recent studies based on machine learning methods have shown that the best risk stratification is achieved by a combination of clinical, genetic and molecular parameters, as well as a combination of clinical parameters with new quantitative Positron Emission Tomography parameters, such as Metabolic Tumor Volume and dissemination features and analysis of circulating tumor DNA levels. This paper provides an overview of studies in which these new risk stratification models were analyzed.

Key words: Lymphoma, Large B-Cell, Diffuse; Rituximab; Risk Assessment; Prognosis; Positron Emission Tomography Computed Tomography; Circulating Tumor DNA; Biomarkers

Sažetak

Uvod. Difuzni B-krupnoćelijski limfom je oboljenje koje se karakteriše patološkom i biološkom heterogenošću i varijabilnim kliničkim ishodom. Zbog izražene heterogenosti prognostički biomarkeri su od velike važnosti u identifikaciji visokorizičnih pacijenata koji bi mogli imati koristi od agresivnijeg lečenja ili novih terapijskih modaliteta. U cilju predviđanja prognoze za pacijente sa difuznim B-krupnoćelijskim limfomom uspostavljeno je i primenjivano nekoliko prognostičkih skoring sistema. Prvi uspostavljeni prognostički sistem za pacijente sa non-Hočkinovim limfomom je internacionalni prognostički indeks, a potom su u eri imunohemoterapije ustanovljene njegove varijacije revidirani *International Prognostic Index* i *International Prognostic Index and National Comprehensive Cancer Network-International Prognostic Index*. Pošto je diskriminativna moć ovih kliničkih prognostičkih skorova suboptimalna, naročito u grupi bolesnika visokog rizika, poslednjih godina postoji tendencija za integracijom genetskih i molekularnih biomarkera i prognostičkih somatskih mutacija u standardizovane i personalizovane modele za stratifikaciju rizika koji bi imali široku primenu u rutinskoj kliničkoj praksi. Rezultati nedavnih studija, zasnovanih na metodama mašinskog učenja, pokazali su da se najbolja stratifikacija rizika postiže kombinacijom kliničkih, genetskih i molekularnih parametara, kao i kombinacijom kliničkih parametara sa novim kvantitativnim parametrima pozitronske emisione tomografije, kao što su zapremina metaboličkog tumora i karakteristike diseminacije, kao i analizom nivoa cirkulišuće DNK tumora. U ovom radu dat je pregled studija u kojima su analizirani ovi novi modeli stratifikacije rizika.

Ključne reči: difuzni krupnoćelijski limfom; rituksimab; procena rizika; prognoza; PET/CT; ctDNA; biomarkeri

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a morphologically, genetically and biologically heterogeneous disease and the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for 35% of NHL cases in Western countries [1]. The standard treatment for all stages of DLBCL is the R-CHOP regimen (rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone), which leads to a cure for 50-60% of patients [2]. Although it is potentially a curable disease, a considerable proportion of patients suffer from a high risk of relapse, despite primary treatment. Thus, it is necessary to find ways for the early identification of patients with a high risk of relapse. Several new prognostic score systems composed of clinical, genetic and molecular parameters have been established and applied to predict the survival of patients with DLBCL.

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Abbreviations

DLBCL – Diffuse B large cell lymphoma
IPI – International Prognostic Index
R-IPI – Revised International Prognostic Index
NCCN – National Comprehensive Cancer Network
MTV – Metabolic Tumor Volume
R-CHOP – rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone
BCCA – British Columbia Cancer Agency
LDH – Lactate Dehydrogenase
ALC – absolute lymphocyte count
AMC – absolute monocyte count
BCL2 – B-cell leukemia/lymphoma 2 protein
XIAP – X-linked inhibitor of apoptosis protein
COO – Cell of origin
GEP – gene expression profiling
GCB – germinal center B-cell
ABC – activated B-cell
IHC – immunohistochemistry
PFS – progression-free survival
DH – Double-Hit
DEL – double expresser lymphoma
MHG – molecular high-grade
ECOG PS – Eastern Cooperative Oncology Group Performance Status
FDG – fluorodeoxyglucose
OS – overall survival
TMTV – metabolic tumor volume
SUV – Standard uptake value
ctDNA – Circulating tumor DNA
NGS – Next-Generation Sequencing
CAR-T – Chimeric antigen receptor T cells

Clinical factors

The first established prognostic score in NHL was the International Prognostic Index (IPI), which has been used since 1993 to predict prognosis in aggressive NHL treated with doxorubicin-based chemotherapy **(Table 1)** [3]. According to IPI values, patients are classified into four risk groups: low, intermediate, intermediate/high and high, with significantly different 5-year overall survival (5-year OS) rates of 73%, 51%, 43% and 26%, respectively. IPI has been validated in the rituximab era and for patients <60 years of age (age-adjusted IPI) [4] **(Table 1)**. The British Columbia Cancer Agency (BCCA) evaluated patients treated with rituximab and CHOP and concluded that a more convenient grouping of risk factors was necessary. Therefore a revised IPI (R-IPI), which yielded three significantly distinct risk groups, was established.

In the rituximab era, a new prognostic model similar to IPI, (NCCN)-IPI was designed. It was developed in an attempt to improve the prognostic capability of the IPI after evaluating data from 1650 DLBCL patients treated with rituximab and chemotherapy in seven National Comprehensive Cancer Network (NCCN) cancer centers **(Table 2)** [6]. It uses the same clinical and biochemical characteristics as IPI, but with stratifying age and LDH [6]. IPI and NCCN-IPI are the two most commonly used prognostic models in routine clinical practice, although they failed to identify the extremely high-risk population fully [7].

In recent years, the prognostic significance of numerous clinical, laboratory and histological parameters has been analyzed, and many novel markers with potential prognostic significance have been identified. Laboratory parameters, such as the ratio of absolute lymphocyte count (ALC), absolute monocyte count (AMC) and histopathologic characters, i.e., BCL2, survivin, XIAP, MYC, and CD5 expression, showed significant impact on clinical outcomes. They were applied to develop new models with improved discrimination power of prognosis [8–14]. Cai et al., established and validated a prognostic nomogram index which consisted of nine clinical and laboratory parameters which performed better than IPI and NCCN-IPI for risk stratification of DLBCL patients [8].

Cell of origin

The Cell of origin **(**COO) classification identifies subtypes originating from B-cells at different developmental stages, with different survival pathways and various outcomes. In 2000, Alizadeh and colleagues used gene expression profiling (GEP) of 96 normal and DLBCL lymphocytes to identify three unique genetic signatures that portended three different subtypes of disease based on COO [15]. These include the germinal center B-cell (GCB)-like subtype, which resembles the GEP of normal GCBs, the activated B-cell (ABC)-like subtype, which resembles normal ABCs, and unclassifiable disease in the remaining 10-15% of samples. Although initially identified by GEP, this assay has had limited clinical utility because of its high cost and the need for fresh frozen tissue [15]. Therefore, alternative strategies were developed for the determination of molecular subtypes, including immunohistochemistry (IHC)

Table 1. Clinical prognostic scores in patients with DLBCL
Tabela 1. *Klinički prognostički skorovi kod bolesnika sa DBK*

	IPI	aaIPI	R-IPI	E-IPI
Age (dob) (≤ 60 ili > 60 years)/*Životna dob (≤ 60 ili > 60 godina)*	+ 60	–	+ 60	+ 70
LDH (normal/elevated)/*LDH (normalan/povišen)*	+	+	+	+
ECOG PS(0-1/> 1)/*ECOG (0–1 ili > 1)*	+	+	+	+
Extranodular disease (0-1/ > 1)/*Ekstranodalna bolest (0-1/> 1)*	+	–	+	+
Number of risk groups/*Broj grupa rizika*	4	3	3	4

Table 2. NCCN IPI
Tabela 2. *NCCN IPI*

NCCN-IPI score/*NCCN-IPI skor*	
Age, years/*dob, godine*	
> 40–≤ 60	1
> 60–≤ 75	2
> 75	3
LDH (LDH)	
> 1 – ≤ 3	1
> 3	2
Ann Arbor stage III-IV/*Ann Arbor stadijum III-IV*	1
Extranodal disease/*Ekstranodalna bolest*	1
Performance status ≥ 2/*Opšte stanje ≥ 2*	1

and, more recently, the NanoString gene expression platform that have shown a greater concordance with GEP than IHC. Multiple studies have shown that patients with the ABC disease subtype have significantly poorer outcomes to standard up-front rituximab-containing chemotherapy compared to GCB disease [16, 17]. In a study of 157 de novo DLBCL cases treated with up-front immunochemotherapy, patients with the ABC subtype, as identified by GEP, had a 5-year progression-free survival (PFS) of 31% compared to 76% in GCB disease, which translated to an inferior 5-year OS (45% vs. 80%) [18]. Similarly, in a study of 344 patients with de novo DLBCL treated with R-CHOP that used the Lymph2Cx assay on the paraffin-embedded tissue to identify COO, the 5-year PFS and 5-year OS were 48% and 56%, respectively, in ABC disease, compared to 73% and 78% in GCB disease [16].

Albitar et al., developed a DLBCL classification method for predicting clinical outcomes using targeted RNA sequencing combined with machine learning algorithms [19]. They developed a strategy that classifies patients with DLBCL into subgroups based on the clinical course of their disease and used modified Bayesian statistics to select genes that can predict various survival groups. This approach uses data from the targeted transcriptome to predict these survival subgroups. Using the expression levels of 180 genes, their model reliably predicted the four survival subgroups and was validated using independent groups of patients [19].

Molecular features

In the past few years, significant achievements have been made in understanding lymphoma biology and exploring the molecular features of DLBCL [20, 21].

The two most important molecular markers in DLBCL are a rearrangement of the anti-apoptotic proto-oncogene BCL2 and/or its transcription repressor BCL6, which are detected in 8–10% of de novo DLBCL patients [22]. Patients with these rearrangements have more aggressive disease and a worse prognosis after frontline treatment with R-CHOP, especially patients with advanced-stage disease [23]. However, even within this group, there is further heterogeneity. More recently, Ennishi et al. performed a comprehensive analysis of RNA sequencing data from 157 patients with GCB DLBCL treated with up-front R-CHOP [24] and established that Double-Hit gene expression signature (DH) is able to identify a high-risk subset of GCB cases (27%). Also, patients with DLBCL can have a double expresser lymphoma (DEL), characterized by over expression of the c-MYC oncogene and BCL2 detected by IHC (≥ 40% and > 50%, respectively). DELs account for approximately a third of de novo cases and have an intermediate prognosis with up-front R-CHOP therapy. DELs can also be detected in up to 50% of relapsed refractory DLBCL, where they are also associated with poorer outcomes with salvage chemotherapy treatment [23]. DH lymphomas are more often registered in GCB subtype, while DE is more often registered in non-GC subtype.

Genetic subtypes

The use of whole-exome sequencing further discovered new genetic subtypes of DLBCL characterized by frequently recurrent mutations. Schmitz et al., analyzed 574 pre-treatment DLBCL biopsy samples, and identified four distinct genetic subtypes of disease with different recurring high-frequency mutations [20]. These categories include the MCD, BN2, N1 and EZB subtypes. The MCD subtype was characterized by the co-occurrence of MYD88 (L265P) and CD79 mutations, the BN2 subtype by BCL62 fusions and NOTCH2 mutations. The N1 subtype had frequent NOTCH1 mutations, and the EZB subtype had EZH2 and BCL2 translocations. The MCD and N1 subtypes corresponded to ABC disease, while the BN2 and EZB subtypes corresponded to the GCB subtype. These groups portend different outcomes to upfront therapy. BN2 and EZB subtypes conferred a good prognosis, while the other subtypes conferred a poor prognosis. The discovery of genetic mutations and subtypes of lymphoma enabled a better understanding of the pathogenesis of lymphoma, better risk stratification, but also the discovery of new therapeutic targets directed at key genetic events **(Figure 1)** [25].

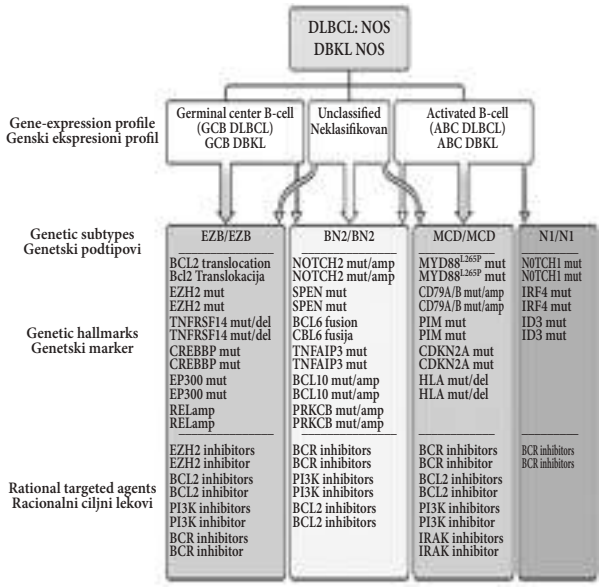

Figure 1. Oncogenic mechanisms and therapeutic targets within genetic subtypes of DLBCL (Roschewski M et al., 2020)
Slika 1. *Onkogeni mehanizmi i terapeutski ciljevi unutar podgrupa DBKL*

Clinico-Genomic Models

Considering the suboptimal discriminative power of clinical scores for OS prediction, there are attempts to define a comprehensive prognostic model incorporating clinic, genetic and molecular parameters. Orgueira et al., (2022) presented a new machine learning model (LymForest-25) based on 25 clinical, biochemical and gene expression variables [26]. LymForest-25 achieved high survival prediction accuracy in patients with DLBCL treated with upfront immunochemotherapy. This model contains 25 variable (19 transcripts, 5 IPI-related variables and the IPI score itself). The validity of the LymForest-25 gene expression signature was confirmed and it achieved substantially greater precision in the estimation of mortality at 6 months and 1, 2, and 5 years compared with the cell-of-origin (COO) plus molecular high-grade (MHG) classification. This model was predictive of survival within the MHG and all COO subgroups, with particularly high accuracy in the "unclassified" group. Integration of this signature with the International Prognostic Index (IPI) score provided the best survival predictions. However, this model showed a limitation of molecular data in the survival prediction of older patients (> 70 y), probably because treatment dose intensity can be largely conditioned by comorbidities and patients' frailty that is not adequately reflected by age and ECOG status [26].

Interim Evaluation

There were many attempts to accurately risk stratify patients prior to and throughout treatment by interim 18F-FDG PET/CT and its integration with IPI.

Several studies found that interim evaluation results from PET-CT or CT scans are predictors of survival. In the study Shi et al., IPI and interim PET/CT were integrated with the aim of improving the stratification efficacy for patients with DLBCL [27]. They established three groups with different OS and PFS rates. This model identified a high percentage of low-risk patients with a favorable prognosis of 3-year OS of > 95% and ~30% of patients with 3-year OS of <5 0%. In some studies, other measurements, different from the commonly used Deauville score, were used in assessing therapy response. For example, in the retrospective evaluation of the 360 patients from the phase 3 REMARC trial, which evaluated the addition of lenalidomide maintenance vs. placebo in DLBCL patients age ≥ 60 years old treated with upfront R-CHOP, the total metabolic tumor volume (TMTV) was used, calculated as the sum of the metabolic volumes of all nodal and extranodal lesions [28]. A high TMVT, defined as > 220 at baseline PET, was able to identify patients with inferior EFS and OS when compared to those with lower TMVT. The prognostic ability of high TMVT was maintained across different treatment groups and after adjustment for LDH, B2-microglobulin, performance status, and clinical risk scores (IPI and NCCN-IPI). Another approach in the assessment of PET/CT is the delta SUVmax which compares the SUV value of the most FDG-avid lesions on the baseline and interim scans and may improve reproducibility during response assessments. Schoder et al., reported the results of a prospective analysis of PET-CT serial evaluations of 504 patients studied in the phase 3 CALGB 50303 trial. They performed a comparison between visual Deauville 5-point scale with percent change in FDG uptake (delta SUV) [29]. With a median follow-up of 5 years, a delta SUV ≥ 66% on interim-PET, measured after two cycles of chemotherapy, was predictive of OS ($p = 0.02$), but not PFS. In contrast, visual assessment by Deauville score did not predict either outcome. The delta SUV value was also assessed in a phase 2 study of 1073 patients with newly diagnosed CD20+ lymphoma, including 609 with DLBCL treated with two cycles of R-CHOP followed by an interim PET CT (iPET) [30]. A negative scan was defined as delta SUVmax > 66%. If the iPET was negative, patients were randomized to R-CHOPx4 arm vs. R-CHOPx4 plus two cycles of rituximab arm. If the interim scans were positive, patients were randomized to an escalated Burkitt protocol arm or R-CHOP × 6 arms. The iPET was negative in 87.5% of patients and positive in 12,5%. The study reported that iPET scan assessed by deltaSUV, but not Deauville score, accurately predicted better 2-year PFS (79.4% vs. 36.7%, $p < 0.0001$) and 2-year OS (88.2% vs. 59% $p < .0001$) in those patients with negative scans across all lymphoma types. However, escalation of treatment based on positive iPET did not translate into improved outcomes, similarly to several earlier trials, demonstrating the limitations of interim PET CT in guiding therapy in DLBCL [31].

Circulating tumor DNA (ctDNA)

Circulating cell-free DNA is continuously released into the peripheral bloodstream by normal or tumor cells undergoing cell death. Novel Minimal Residual Disease strategies use next-generation sequencing (NGS) techniques to identify clonal tumor immunoglobulin heavy chain sequences or tumor-specific mutations from a panel of disease-specific genes - cancer personalized profiling by deep sequencing [32]. The advantages of monitoring cfDNA are its non-invasive nature with the potential to follow clonal evolution and detect new mutations that arise during treatment, which could target new therapeutic agents. In a landmark study, Rochewski and colleagues retrospectively analyzed ctDNA in pretreatment tumor specimens, and serial serum samples of 126 patients with untreated DLBCL enrolled in three trials of upfront R-EPOCH vs. EPOCH [33]. CtDNA was analyzed using NGS by clonal VDJ rearrangements. After completion of treatment, patients were monitored with serial CT scans and concurrent serial serum samples. With a median of 11 years, positive ctDNA during surveillance had a positive predictive value of 88.2% and a negative predictive value of 97.8% for relapse. Patients developed detectable ctDNA with a lead time of 3.5 months prior to clinical progression. The ability of circulating tumor DNA (ctDNA) to detect early relapse has since been confirmed in several other studies, including in high-risk patients, post-allo-HSCT, and in the RR setting in patients receiving CAR-T therapy or other novel therapies [33].

When molecular response, measured by ctDNA levels, is combined with interim PET/CT, it is possible to identify a subset of patients with an extremely high risk of treatment failure, which may allow for earlier intervention or alternative therapies, for example, autologous bone marrow transplantation or chimeric antigen receptor T cells (CAR-T) [33]. Current and emerging PET/CT parameters will likely need to be factored into an algorithm with clinical factors and molecular markers, both at baseline and throughout treatment as dynamic response markers.

Conclusion

Over the past decade, numerous genetic and molecular biomarkers have been proven to be prognostic or predictive. However, incorporating these biomarkers and clinical factors in a single risk stratification system remains challenging. The main issue with new prognostic models is that they are very complicated, require expensive methods and a lot of time, and therefore it remains to be seen which of these models will be applied in routine clinical practice.

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HIGH-GRADE B-CELL LYMPHOMA

AGRESIVNI KRUPNOĆELIJSKI B-LIMFOMI

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Summary

Introduction Aggressive B-cell lymphomas are a heterogeneous group of diseases with various clinical, patohistological, genetic characteristics and a variety of treatment outcomes. Diffuse large B-cell lymphoma is the most common lymphoma in European countries, some lymphomas are recognized as specifically aggressive, providing non-adequate response to the standard treatment (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone). **High-grade B-lymphomas.** One group consists of those which are carriers of the c-myc, bcl-2 or bcl-6 rearrangement established by Fluorescence in situ hybridization, and are called high grade B lymphomas, which can be double hit or triple hit. The other group consists of those with blastoid morphology, which are not carriers of the c-myc, bcl-2 and bcl-6 rearrangement and are called Not Otherwise Specified. This heterogeneous group is a carrier of a single myc mutation in 45% of cases, in 15% of patients, an additional analysis of gene expression profiling indicates the presence of high grade B lymphomas double hit sig+. Extranodal localization with Central Nervous System involvement is frequently reported. **Clinical staging and disease progression along with risk assessment by means of IPI scores and aaIPI scores in patients with high-grade B-lymphomas.** Diagnostic procedures during the clinical interview, physical examination, laboratory analyses and various additional diagnostic procedures. **High-grade B-lymphomas treatment.** Studies indicate more intensive induction chemotherapy including central nervous system prophylaxis for these patients. **Conclusion.** The question of how to choose the most effective therapeutic strategy in high grade B lymphomas is still open at this moment, and examinations are focused on the research of molecular mechanisms of lymphomagenesis.

Key words: Leukemia, Lymphocytic, Chronic, B-Cell; Diagnosis; Therapeutics; Risk Assessment; Genetics; Classification

Sažetak

Uvod. Agresivni B-ćelijski limfomi su heterogena grupa oboljenja sa različitim kliničkim, patohistološkim, genetskim karakteristikama i različitim ishodom lečenja. U grupi difuznog B-krupnoćelijskog limfoma kao najučestalijeg u zemljama Evrope, prepoznaju se neki entiteti sa posebnom agresivnošću i neadekvatnim odgovorom na standardno lečenje (rituksimab, ciklofosfamid, doksorubicin, vinkristin, prednizolon). ***High grade* B-limfomi.** Jednu grupu čine oni koji su nosioci c-myc i bcl-2 i /ili bcl-6 rearanžmana utvrđenog fluorescentnom *in situ* hibridizacijom i nazivaju se *High grade* B-limfomi, a u zavisnosti od broja mutacija mogu biti double ili triple hit. Drugu grupu čine oni sa blastoidnom morfologijom koji nisu nosioci c-myc, bcl-2 i bcl-6 rearanžmana i nazivaju se *Not Otherwise Specified high grade* B-limfomi. Ova grupa je heterogena, u 45% slučajeva je nosilac *single myc* mutacije, a u 15% dodatna analiza (engl. *gene expression profiling*) ukazuje na *high grade* B-limfome, double hit lymphoma signature +. Česta je ekstranodalna lokalizacija sa zahvatanjem centralnog nervnog sistema. **Određivanje stadijuma bolesti i procena rizika *IPI* skor *aaIPI* skor u *High grade* limfomima.** Dijagnostičke procedure u obradi bolesnika podrazumevaju adekvatnu anamnezu, fizikalni pregled, laboratorijske analize i različite dodatne imidžing dijagnostike. **Lečenje *high grade* B-limfoma.** Studije predlažu intenzivniji hemioterpijski indukcioni tretman za ove bolesnike uz profilaksu centralnog nervnog sistema. **Zaključak.** *High grade* B-limfomi ostaju veliki terapijski izazov. Pitanje izbora najoptimalnije terapije u ovom trenutku ostaje otvoreno. Ispitivanja su usmerena na istraživanje molekularnih mehanizama limfomageneze. Ispitivanja su usmerena na istraživanje molekularnih mehanizama limfomageneze.

Ključne reči: agresivni krupnoćelijski B limfomi; dijagnoza; terapija; procena rizika; genetika; klasifikacija

Introduction

Non-Hodgkin lymphomas (NHL) are a heterogeneous group of malignant lymphoid tumours, arising from B cells or T cells, that is, NK cells, remaining at various stages of differentiation [1]. A majority (85%) of NHLs are of B-lymhocyte origin, whereas the remaining 15% of NHLs are of T-cell or natural killer (NK)-cell origin and a mix of B-cell and T-cell origin. Regular lymphocytes, as carriers in the immune response, continually receive both cell survival and cell death signals, while their balance enables homeostasis and prevents the development of autoimmunity. But, for unknown rea1sons, a loss of cell cycle regulation of the abovementioned processes leads to neoplastic alteration and uncontrolled proliferation. With regards to the incidence of this type of lymphomas, it is the fifth most common cancer after lung, prostate, breast and colon cancer. The incidence of non-Hodgkin lymphoma has doubled over the past two decades (19.7%) unlike other lymphoproliferative diseases, the incidence of

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Abbreviations
HGBL – High-grade B-cell lymphoma
NOS – Not Otherwise Specified
FISH – Fluorescence *in situ* hybridization
DHIT – Double-hit lymphoma
THIT – Triple-hit lymphoma

which has remained almost the same [2, 3]. Diffuse large B-cell lymphoma is the most common type of lymphoma, comprising a large number of various entities. Due to new genetic markers along with clinical and immunophenotypic features between lymphoid neoplasmas, the new World Health Organization (WHO) classification of lymphoid neoplasmas was introduced in 2016 [4]. Approximately 40% of all B-cell lymphomas are characterized by the presence of recurrent chromosomal translocations and most of them can easily be detected by the use of conventional cytogenetics (karyotyping) or molecular cytogenetics (for example, fluorescence *in situ* hybridization (FISH)) [5, 6].

The c-Myc proto-oncogene (Myelocytomatosis Viral Oncogene Homolog) is localized at q24 domain of chromosome 8 (8;q24). It consists of three exons encoding synthesis of three different protein products with a molecular mass of 64, 67 and 45 kDa. It was discovered almost fifty years ago as the cellular homology of the avian myelocytomatosis viral oncogene (v-Myc). It belongs to the four-member family of myc genes. Of the Myc family members, only one member (v-Myc) has no ability to undergo malignant transformation, whereas the remaining three members (L-Myc, N-Myc and c-Myc) have this particular ability. C-Myc is a nuclear protein playing its own role as a transcription factor, regulating many genes involved in cellular differentiation, proliferation and programmed cell death. Well-known mechanisms of damage of c-myc proto-oncogenes are chromosomal translocations, provirus insertions, gene amplifications and gene mutations. Each of the given examples of c-myc proto-oncogene damage may produce similar effects, such as the alterations in the transcription levels along with the ones detected in the level of mRNA stability. All types of c-Myc gene-related chromosomal abnormalities are most commonly observed in the group of malignancies associated with poor prognostic outcomes. The largest incidence rates of c-Myc gene-related amplification are described in the research of breast, ovarian, gastric and colon cancer, from the range of hematologic malignancies such as multiple myeloma and NHL. In such cases, c-Myc proto-oncogene expression levels almost always positively correlate with the advanced disease stages, due to which c-Myc proto-oncogene is qualified as a negative prognostic parameter [7–9]. The translocation t(8;14)(q24;q32) is the first characteristic chromosomal aberration the presence of which is shown to be associated with the lymphoproliferative diseases [10]. It results in the over-expression of the c-Myc protein, which has not only a transcriptional role, but an important role in cell metabolism as well, protein synthesis, apoptosis regulation and induction by activating p53 or pro-apoptotic signal pathways [11–13]. It is the most common form of transcription characteristic of Burkitt lymphoma (BL), whereas the c-Myc translocations can be proven in approximately 5–10% of all patients with NHL DLBCL, just like in patients with non-classified B-cell lymphoma with features intermediate between Burkitt lymphoma (BL) and NHL DLBCL, plasmablastic (PBL) and lymphoblastic lymphoma, that is, acute lymphoblastic leukemia (ALL) [14, 15].

The Bcl-2 gene (B-cell CLL/lymphoma 2 (BCL2)) is located on chromosome 18 band q21 (18 q21). It is a member of BCL-2 family consisting of 25 not yet identified genes that encode synthesis of proteins responsible for regulation control of programmed cell death (PCD), that is, apoptosis induced via the mitochondrial pathway. The internal mitochondrial pathway of apoptosis is initiated by irreparable DNA damage, caused by replication errors, non-ionizing radiation effects, chemotherapeutics, drugs such as nucleoside analogs along with numerous DNA reactive toxins, including reactive oxygen radicals as well [16–18].

All products of the Bcl-2 family of genes participate in apoptosis, and are most frequently recognized as major regulators of the permeability of the mitochondrial membrane. Depending on their own role in the apoptotic chain, members of the Bcl-2 family can be both pro- and anti-apoptotic. Some of the anti-apoptotic proteins include: Bcl-2, Bcl-x, Bcl-xl, Bcl-xs, Bcl-w, Bag, whereas some of

Table 1. Risk stratification – the IPI score for patients older than 60 years
Tabela 1. *Stratifikacija rizika – IPI skor za bolesnike starije od 60 godina*

Age *Godine*	≥ 60 years/*godina*	1 Negative point *1 Negativan poen*
The Eastern Cooperative Oncology Group (ECOG) performans status/*Opšte stanje bolesnika*	≥ 2	1 Negative point *1 Negativan poen*
Increased (LDH) value *Povišena vrednost LDH*	>1 x within the normal range of values *Povišena vrednost*	1 Negative point *1 Negativan poen*
Ann Arbor clinical stage *Klinički stadijum po Ann Arbor klasifikaciji*	III and IV/*III i IV*	1 Negative point *1 Negativan poen*
Extranodal sites of the disease *Ekstranodalna mesta bolesti*	≥ more than one localization/*više od jedne lokalizacije*	1 Negative point *1 Negativan poen*

Table 1a. Risk categories based on the total IPI score
Tabela 1a. *Stepen rizika u zavisnosti od IPI skora*

Low risk/*Nizak rizik*	0 or/*ili* 1
Low-intermediate risk/*Srednje nizak rizik*	2
High-intermediate risk/*Srednje visok rizik*	3
High risk/*Visok rizik*	4 or/*ili* 5

the pro-apoptotic ones include: Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik and Blk [17, 18]. The pro-apoptotic proteins bind to the outer mitochondrial membrane (OM), which then alter the integrity of the OM, eventually leading to an increase in the OM permeability. This is followed by entry of calcium ions and subsequent release of various proteins from mitochondria, the most important of which is cytochrome c. After the release of mitochondrial cytochrome c into the cytoplasm, cytochrome c forms part of a proteinaceous complex that directly activates caspase-9, which in turn drives the formation of the apoptosome along with cytochrome c. The result of such a polymerase chain reaction is the activation of an effector protein, caspase-3, that causes the degradation of apoptotic cells, which entails the cleavage of cytoskeletal actin, cellular growth and apoptotic bodies being formed through a process termed apoptotic cell disassembly, after which they are phagocytized by neighbouring cells with no inflammatory reaction [17–19]. The most significant Bcl-2 family member and the subject of the most research is the Bcl-2 gene. There are two isoforms of the Bcl-2 protein, which are considered to play the role in at least two different anti-apoptopic mechanisms [20] due to their specific interaction with Bad and Bak proteins. This particular translocation is present not only in NHL DLBCL, but also in other lymphoproliferative diseases which are of B-lymphocyte origin, such as follicular lymphoma, primary cutaneous lymphoma, primary cutaneous marginal zone lymphoma (PC-MZL), chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), splenic lymphoma and histiocytic sarcoma (HS) arising from the follicular lymphoma transformation. The presence of damage to the Bcl-2 gene has been proven to be found in approximately 90% of colon cancer cases, 60% of breast cancer cases, 30% of prostate cancer cases and small cell lung cancer (SCLC) cases, whereas, with regards to the variable incidence, it has been observed to be the case with other malignancies of the gastrointestinal tract, thyroid gland, etc. In all the aforementioned tumors, Bcl-2 rearrangement results in the overexpression of proto-oncogene Bcl-2 [21–24]. Overall, apart from playing a significant role in lymphomagenesis and carcinogenesis, the presence of Bcl-2 translocation has been reported in healthy individuals as well. The persistent clone of normal mature B-cell lymphocytes with the aforementioned translocation is most frequently reported in the older population group [25]. The presence of translocation in healthy individuals, just like its relatively early occurrence during the B-cell lymphocyte maturation, indicates not only the importance of role of Bcl-2 translocation in lymphomagenesis, but it also leads to a conclusion that the presence of translocation itself is not considered to be a sufficient factor for the development of lymphproliferative disease [26]. The World Health Organization (WHO) classification emphasizes how important it is to determine the Bcl-2 translocation prior to diagnosing aggressive B-cell lymphomas – double-hit lymphomas, which should be differentiated from NHL DLBCL not otherwise specified (NOS) [27].

The Bcl-6 gene (B-cell CLL/lymphoma 6 (BCL6)) is located on chromosome 3 band q27 (3q27), encoding protein synthesis, which is a transcription factor playing an important role in the transcription repression due to its interaction with cell proteins. In addition, it is involved in the repression of genes significant in terms of the activation and differentiation of lymphocytes, cell cycle regulation and inflammation [28, 29]. The Bcl-6 gene expression is of a particular significance for the formation and normal functioning of germinative centres, and its own role extends to the prevention of DNA double-stranded break-induced apoptosis in B-lymphocytes [29]. Additionally, the Bcl-6 gene participates in the cell cycle regulation, in various DNA reparation mechanisms, apoptosis, cell proliferation and differentiation [30, 31]. Besides, it has a direct impact on the regulation of numerous genes, such as the following: Bcl-2 and p53 [32, 33]. Chromosomal aberrations involving Bcl-6 in shape of translocations and point mutations are most commonly reported in patients with NHL DLBCL and follicular lymphoma in particular. Bcl-6 translocation with some of the numerous potential partner genes is regarded as a feature of *double-hit* lymphoma [34]. Unlike the Bcl-1 and Bcl-2 genes, that always sit in close proximity compared to the genes encoding protein synthesis of both the heavy and light chains (IgH and IgL) after the chromosomal fragments translocation is ended, the Bcl-6 gene is localized in the vicinity of various gene sequences. The translocation includes chromosome 3 and RhoH (TTF gene) gene, chromosome 11 ((BOB 1/OBF 1 as a "partner" gene), chromosome 6 (H4 histone gene lying in close proximity), chromosome 13 (LCP 1 gene lying in close proximity), and a plenty of other non-immunoglobulin genes. The physiological function of Bcl-6 gene is its participation in the formation of germinative centres of secondary lymphoid follicles, just like in other sites in

Table 2. Risk stratification – The age-adjusted IPI for patients under the age of 60
Tabela 2. *Stratifikacija rizika – age adjusted IPI skor za bolesnike mlađe od 60 godina*

Ann Arbor clinical stage *Klinički stadijum po Ann Arbor klasifikaciji*	III and IV/*III i IV*	1 Negative point *1 Negativan poen*
Values LDH *Vrednost LDH*	> 1 x within the normal range of values *Povišena vrednost*	1 Negative point *1 Negativan poen*
The Eastern Cooperative Oncology Group performans status/*Opšte stanje bolesnika*	from 2 to 4/*od* 2 *do* 4	1 Negative point *1 Negativan poen*

which B-cells reach maturation. The second important role of Bcl-6 gene is related to preventing the process of a gene's DNA sequence being copied (transcribed), just like the regulation of programmed cell death by means of the adhesion molecule CD 40. Chromosomal rearrangements including Bcl-6 occur in various percentages. With regards to NHL and DLBCL, alterations in the level of Bcl-6 occur in 49% to 61% of patients [35]. In the germinative centre, Bcl-6 plays a role in the expression of numerous target genes that participate in apoptosis, just like the expression of genes accounting for the cell cycle regulation, proliferation and differentiation [36, 37]. Bcl-2, p53, IRF4 and BLIMP-1 are considered important direct targets [38, 39]. Interestingly, BLIMP-1 repressor is simultaneously Bcl-6 and c-Myc in the plasma cells, the result of which is facilitated Bcl-6 of the p53-mediated repression, somatic hypermutation (SHM) and class-switch DNA recombination (CSR) [40].

High-Grade B-cell Lymphoma

The association of Bcl-2+/c-Myc+ gene rearrangement accounts for the majority of *double-hit* lymphomas (even 62%), according to the data obtained by the *Mittelman Database* [41, 42]. The c-Myc gene translocation, which participates in numerous cell functions, including proliferation as well, indicates poor prognosis [43–45]. With the addition of the Bcl-2 translocation, which is considered as the central anti-apoptotic gene, it affects the outcome in some studies, but not in all of them [46, 47]. NHL DLBCL with the translocation of both c-Myc and Bcl-2 genes – determines double-hit lymphomas and is characterized by a poor response to standard therapy with an aggressive clinical course [48–50]. When combined together, these two genes have a synergistic clinical effect: c-Myc as a cellular proliferation regulator and Bcl-2 as a blocker of programmed cell death and apoptosis. Nowadays, research studies of c-Myc and Bcl-2 combinations found in lymphomas – focus on genetic methods, mostly fluorescence in situ hybridization. However, c-Myc and Bcl-2 can be activated by means of other mechanisms, carrying an increased c-Myc and Bcl-2 protein expression [51, 52]. Summing up the basic concept within the classification of aggressive B-cell lymphomas complete with new findings has lead to the emergence of a new concept called High-grade B-cell lymphoma (HGBL) entity, replacing the non-classified NHL DLBCL group, which was, in turn, renamed as the "gray zone" lymphoma denoting a type between NHL DLBCL and "Burkitt" lymphoma. HGBL is a carrier of the c-Myc translocation complete with Bcl-2 and/or Bcl-6 rearrangement (HGBL – *Double-Hit*) or (HGBL – *Triple-Hit*). NHL DLBCL NOS (not otherwise specified) with blastoid morphology without the Myc and Bcl-2 and/or Bcl-6 rearrangement – is also classified into this particular group [53]. An immunohistochemistry (IHC) panel used in the diagnosis of HGBL shows positivity for: CD20, BCL6, CD10, MYC, BCL2, Ki67, IRF4/MUM1, Cyclin D1, CD5 and CD 23 [54]. Genetic analyses are characterized by the clonal rearrangements of immunoglobulin genes, demonstrating that 20% of patients report the presence of the Bcl-2 rearrangement, 10% of patients report the c-Myc rearrangement, 30% of patients report the Bcl-6 rearrangement, whereas 70% of patients with NHL DLBCL report the mutations of Bcl-6 gene. The presence of the Bcl-2, Bcl-6 and c-Myc rearrangement is established by means of the *Fluorescence in situ hybridization* (FISH) technique, because immunohistochemical expression only partially correlates with the presence of chromosomal aberration. FISH is a molecular cytogenetic technique based on the hybridization of gene sequences of the examined chromosome and an adequate series of nucleotides bound to the fluorescent probes. *In situ* hybridization is a technique which enables the detection and localization of specific nucleic acids, that is, the detection of specific target sequences within an individual cell with a simultaneous preservation of cellular and tissue morphology. The principle of *in*

Table 2a. Risk assessment categories for patients under the age of 60
Tabela 2a. *Kategorije procene rizika za bolesnike mlađe od 60 godina*

Low risk/*Nizak rizik*	0
Low-intermediate risk/*Srednje nizak rizik*	1
High-intermediate risk/*Srednje visok rizik*	2
High risk/*Visok rizik*	3

situ hybridization (ISH) is based on complementarity, meaning that the probe specifically hybridizes to its complementary target sequence on the nucleic acids (DNA or RNA). The term in situ means "in the original place or position", meaning that hybridization takes place within an individual cell, but specifically in the place where the target DNA or RNA is normally located. The targeted region can be a gene, chromosome region or the whole chromosome. Due to FISH, it is possible not only to analyze a fresh cell sample which was previously fixed, but even the formalin-fixed paraffin-embedded biopsy tissues as well. A question is being raised as to whether or not FISH analysis is the gold standard used for the purpose of the HGBL identification with the Myc, Bcl-2 and/or Bcl-6 rearrangement, since, in light of new findings of numerous complex genome research, it is suggested that FISH analysis is limited by the detection sensitivity compared to the techniques such as whole-exome sequencing (WES) [55]. The examination of numerous cases of DHL/THL in high-grade B-cell lymphomas with critical aberrations shows that they have not been identified by using the FISH method. It means that double-hit gene expression signature (DHIT-sig+) is defined based on the analysis of RNA sequencing data. Several studies indicate that the Myc and Bcl-2 rearrangement is identified in only half of the patients with DHIT-sig+, but they also emphasize that these patients make progress in a shorter time interval compared to those identified as DHIT. To the contrary, some studies demonstrate that the association of DHIT sig+ and tp53 abnormalities in patients with HGBL leads to poor prognosis and low overall survival (OS) [27, 56].

Clinical staging and disease progression along with the risk assessment by means of IPI scores and aa IPI scores in patients with HGBL

Diagnostic procedures during the clinical interview with patients with HGBL, encompass adequate medical history taking (anamnesis), physical examination with laboratory analyses and various additional diagnostic procedures. By using the abovementioned diagnostic procedures, it is possible to identify the most adequate location for the biopsy of tumor tissue that is to be subjected to a histopathological analysis, immunophenotypization, classical cytogenetic analysis or molecular genetic analysis (fluorescence in situ hibridization – FISH). The HGBL diagnosis requires additional procedures to be undertaken such as the following: lumbar puncture and liquor analysis by using flow cytometry with immunophenotypization, in case of the need to perform nuclear magnetic resonance (NMR) imaging of the CNS. In order to adequately assess the clinical stage of the patient, blind bone biopsy is performed. The Ann Arbor staging classification was initially adopted for Hodgkin's disease in 1971, but it is also applied for NHL DLBCL, and even for HGBL. The classification describes four clinical disease stages. Special emphasis is placed on extranodal localization with an addition of the capital letter E. Special consideration is given to cases of bulky disease, simultaneously pointing out large tumor mass [57]. Each of the patients is assigned with an *International Prognostic Index* (IPI) risk category. The IPI score is established for patients over 60, whereas, for those under the age of 60 – the age-adjusted IPI (aaIPI) score is used. The IPI values and scoring system are shown in **Table 1** and **1a**, and also in **Table 2** and **2a**.

IPI is a useful clinical method for classifying patients into differentiated risk groups. However, it does not take into consideration a genetic spectrum of their varieties. Patients with HGBL are often presented with poor prognostic parameters, elevated LDH values, bone marrow and CNS involvement complete with high IPI score [58].

High-Grade B-cell Lymphoma treatment

Previously mentioned diagnostics, complete with the assessment of disease extent and prognostic assessment of patients and their clinical profiling, undoubtedly raise a question as to how to treat patients diagnosed with HGBL. Numerous retrospective observational studies have shown that standard treatment performed according to the R-CHOP regimen in patients who are carriers of gene mutations (such as c-Myc, Bcl-2 and/or Bcl-6), results in significantly poorer survival rates when compared to patients with NHL DLBCL who are not carriers of such aberrations. Thereby, the study conducted by Rosenwald et al., which analyzed 2.383 respondents who received R-CHOP chemotherapy – demonstrated that PFS and OS were shorter in patients who were carriers of the c-Myc rearrangement associated with Bcl-2 and/or Bcl-6 [59]. Dunlevy et al., conducted a prospective multicentric study that comprised 53 patients with aggressive B-cell lymphomas, 44% of which had the c-Myc rearrangement (SH), whereas 56% of patients (with DHL/THL) who were treated with DA-EPOCH-R, reported no statistically significant difference in EFS and OS (71% vs. 77%) between SH and DHL/THL during the four-year observation period. However, the fact that it was a small sample size must be taken into account [60]. The French retrospective study carried out by Lauda et al., which comprised 160 patients with HGBL (81% DHL and 19% THL) showed a significantly higher PFS compared to those who received intensive chemotherapy as opposed to R-CHOP [61]. The standard therapeutic regimen has not been established. Considering the fact that retrospective studies reveal no data of intensive chemotherapy as being superior, these patients should be involved in clinical trials, the subject of which is the examination of targeted therapies supporting the key mechanism of pathogenesis of Myc and Bcl-2 activation. On one hand, intensive chemotherapy is administered in patients who are able to receive such a therapy, particularly the ones with a high IPI score (a personalized approach from DA-EPOCH-R to R-Hyper-CVAD and R-IVAC), but on the other hand, with regards to the patients who are not considered as

eligible candidates for high-dose chemotherapy or TMHC due to the presence of comorbidities or advanced age – we return to the treatment with R-CHOP, R-COEP, and then to the treatment of patients with relapsed/refractory DLBCL (RCEPP, Polatuzumab Vedotin+Rituximab+Bendamustine), radiation therapy as a local treatment modality, the involvement of patients in the clinical trials, eventually turning to the best supportive care as the worst possible option. The role of CNS prophylaxis in the treatment of patients with HGBL remains controversial. However, patients with a high IPI score, extended disease and bone marrow infiltration, can benefit from the implementation of intrathecal therapy (MTX and AraC/4 – 8 doses) or systemic CNS prophylaxis (MTX i.v. in 2 – 4cy) [62]. Monitoring the patient's response by applying PET/CT imaging is by all means indicated.

Conclusion

HGBL at diagnosis remains a major therapeutic challenge. The question of how to choose the most effective therapeutic strategy is still an open one. Of key importance is the further investigation of underlying pathophysiological mechanisms of this particular group of lymphoproliferative diseases, along with the understanding of interactions between genetic alterations and lymphomagenesis. In that respect, the identification of new genetic signatures, such as the previously mentioned DHIT sig+ is very important knowledge, just like the revelation of new c-Myc and Bcl-2 gene interactions and dysregulations. If there is "*double expressor*" defined by the co-expression of c-Myc and Bcl-2 by using immunohistochemistry (IHC) and if the morphology of the disease responds to NHL DLBCL GC, then it is necessary to perform FISH by means of which it is possible to detect HGBL. It is evident that the R-CHOP protocol is not regarded as the most adequate therapeutic choice for a group of patients with HGBL. Some of these patients can benefit from a more intensive therapy, but it requires a better randomization in clinical trials and conducting prospective cohort studies as well. A number of ongoing research studies seem promising, primarily the ones targeting at the c-Myc and Bcl-2 genes. A series of recent studies has indicated the importance of implementing new treatment strategies, such as the application of newly approved anti-CD19 monoclonal antibodies and chimeric antigen receptor (CAR) T-cells in the treatment of patients diagnosed with high-risk NHL DLBCL and HGBL, who do not achieve a complete clinical response to the previously applied therapy.

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RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA – ADVANCEMENTS IN TREATMENT

RELAPSIRAJUĆI/REFRAKTORNI DIFUZNI B-KRUPNOĆELIJSKI LIMFOM – POMACI U LEČENJU

Olivera TARABAR

Summary
About 40% of patients with diffuse large B-cell lymphoma will develop disease relapse or refractory disease to first-line rituximab-cyclophosphamide, doxorubicin, vincristine and prednisone chemotherapy, necessitating second-line therapy. Historically, this consisted of platinum-based chemotherapy followed by autologous stem cell transplantation for patients who were transplant-eligible. But not all patients are eligible for autologous stem cell transplantation and curative treatment options for these patients were limited. The lack of effective treatment options in the relapsed/refractory diffuse large B-cell lymphoma had made the prognosis of these patients poor. In recent years there have been several new therapeutic agents approved or pending approval for the treatment of relapsed/refractory diffuse large B-cell lymphoma. These treatments include antibody-drug conjugates, novel anti CD19 monoclonal antibodies, chimeric antigen receptor T-cell therapy, bispecific antibodies, and selinexor. This paper reviews current strategies and novel therapies for relapsed/refractory diffuse large B cell lymphoma.
Key words: Lymphoma, Large B-Cell, Diffuse; Therapeutics; Antineoplastic Combined Chemotherapy Protocols; Recurrence; Prednisone; Doxorubicin; Vincristine; Cyclophosphamide; Rituximab

Sažetak
Približno 40% pacijenata sa difuznim B-krupnoćelijskim limfomom će relapsirati ili ispoljiti refraktornu bolest na prvu liniju terapije po rituximab-cyclophosphamide, doksorubicin, vincristine i prednisone protokolu zahtevajući terapiju druge linije. Godinama unazad, ona se sastojala od hemioterapije bazirane na preparatima platine i autologne transplantacije matičnih ćelija hematopoeze kod pacijenata koji su pogodni za transplantaciju. Međutim nisu svi pacijenti kandidati za autolognu transplantaciju matičnih ćelija hematopoeze i opcije lečenja sa kurativnim potencijalom za ove pacijente su bile ograničene. Nedostatak efikasnih terapija u lečenju relapsirajućeg/refraktornog difuznog B-krupnoćelijskog limfoma doprineo je da je prognoza ovih bolesnika bila nepovoljna. Poslednjih godina, nekoliko novih terapija su odobrene ili čekaju odobrenje za lečenje relapsirajućeg/refraktornog difuznog B-krupnoćelijskog limfoma. Ove terapije uključuju antitelo-lek konjugate, nova anti-CD19 monoklonska antitela, terapija himeričnim antigen-receptor T-ćelijama, bispecifična antitela i selinexor. Ovaj rad iznosi aktuelne strategije i nove terapije za relapsirajući/refraktorni difuzni B-krupnoćelijski limfom.
Ključne reči: difuzni B-krupnoćelijski limfom; terapija; protokoli kombinovane antineoplastične terapije; relaps; prednizon; doksorubicin; vinkristin; ciklofosfamid; rituksimab

Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common high grade non-Hodgkin lymphoma in adults. Ever since the addition of the anti CD20 monoclonal antibody rituximab to CHOP chemotherapy (R-CHOP) it has lead to a marked improvement in survival of patients with newly-diagnosed DLBCL. However, about 30% of patients relapse after achieving a complete response and 10% are refractory to initial immunochemotherapy [1].

Platinum-based salvage chemotherapy followed by consolidation with high-dose therapy and autologous stem cell transplantation (ASCT) is the standard of care for eligible patients with relapsed DLBCL [2]. However, approximately 50% of patients with relapsed/refractory DLBCL are not candidates for potentially curative ASCT approaches, mainly because of advanced age, comorbidities or inadequate response to salvage chemotherapy [3]. Furthermore, there is a large portion of patients that will experience relapse after ASCT, whose prognosis will, unfortunately, be poor.

A recent retrospective meta-analysis of patients with refractory DLBCL showed an objective response rate (ORR) of 26% to the next line of therapy with average overall survival (OS) of 6,3 months [4].

Currently there is no defined standard of care in this setting and common therapeutic options include adjusted chemotherapy according to the frailty status of the patient, radiotherapy for localized lesions or optimal supportive care. Allogeneic stem cell transplantation (SCT) may be considered for a small proportion of medically-fit patients who relapse after ASCT.

In recent years, there have been major advancements in therapeutic options, namely, antibody-drug conjugates (ADCs), novel CD19 monoclonal antibodies, anti CD19 chymeric antigen receptor (CAR) T-cells, bispecific antibodies, and selinexor.

Antibody-drug conjugates

Polatuzumab vedotin and loncastuximab tesirine belong to a class of antibody-drug conjugates (ADCs).

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Abbreviations

DLBCL	– Diffuse large B-cell lymphoma
R-CHOP	– Rituximab-cyclophosphamide, doxorubicin, vincristine and prednisone
ASCT	– Autologous stem cell transplantation
ORR	– Objective response rate
OS	– Overall survival
ADCs	– Antibody-drug conjugates
CAR T-cell	– Chimeric antigen receptor T-cell
MMAE	– Monomethyl auristatin E
Pola-BR	– Polatuzumab vedotin-bendamustine and rituximab
CR	– Complete response
PFS	– Progression free survival
DoR	– Duration of response
BAs	– Bispecific antibodies
CRS	– Cytokine release syndrome
ICANS	– Immune effector cell-associated neurotoxicity syndrome
SINE	– Selectve inhibitor of nuclear export

Polatuzumab vedotin is an anti CD79b monoclonal antibody conjugate to monomethyl auristatin E (MMAE), which is a potent antimitotic agent. Following the intake of polatuzumab vedotin into the B-cell, MMAE is released, exhibiting its cytotoxic effect.

Recently, polatuzumab vedotin combined with bendamustine and rituximab (pola-BR) was compared with bendamustine and rituximab (BR) in a phase 2 randomized trial of patients (40 pts in each arm) with transplant-ineligible relapsed/refractory DLBCL. Patients were excluded from the study if they had prior ASCT, primary-refractory or double/triple hit lymphomas. The complete response (CR) rate for pola-BR was significantly higher (40% vs. 17.5%) compared with BR. After an average follow-up of 22.3 months, average progression free survival (PFS) (9.5 vs. 3.7 months) and average OS (12.4 vs. 4.7 months) were significantly improved with pola-BR vs. BR. The results of this study favored pola-BR regardless of age, IPI score, and cell of origin (ABC vs. GCB) [5].

Results from randomized trials as well as real-world data for polatuzumab vedotin have been promising. It is important to mention that real-world data has shown a comparable response rate, however, PFS and OS were shown to be inferior to those demonstrated in phase 2 randomized trials. This can be attributed to more heavily pretreated patients in real-world care [6, 7].

Toxicity profile of pola-BR consisted mainly of cytopenias, peripheral neuropathy, and less commonly, respiratory tract infections [5].

On the basis of the results from the study by Sehn et al., polatuzumab vedotin in combination with bendamustine and rituximab (pola-BR) has been approved for use in adult patients with relapsed or refractory DLBCL ineligible for ASCT.

Promising response rate and durability of responses in patients treated with pola-BR in relapsed/refractory DLBCL have proven that pola-BR may be used as a bridge treatment to ASCT or CAR T-cell therapy. In a study by Liebers et al., polatuzumab vedotin and rituximab (without bendamustine) were administered as bridging therapy to 41 patients who were to receive CAR T-cell; half of patients successfully underwent CAR T-cell treatment with a 6-months and 12-months OS of 78% and 58.5%, respectively [8].

Furthermore, loncastuximab tesirine is a novel anti CD19 ADC in the treatment of relapsed/refractory DLBCL. Initial results of a phase 2 clinical study (LOTIS-2) of loncastuximab tesirine in 145 patients with relapsed/refractory DLBCL are encouraging. The ORR was 48.3% and CR was 24.1% with a median duration of response of 12.6 months. The treatment with loncastuximab tesirine is associated with moderate cytopenias, pleural/pericardial effusions, and edemas [9]. Results of this study have shown that loncastuximab tesirine can be an effective therapy option in patients relapsing after ASCT or CAR T-cell therapy with ORR of 58.3% and 46.2%, respectively.

CD19 Monoclonal Antibodies

Tafasitamab is a humanized anti CD19 monoclonal antibody that, when combined with lenalidomide, produces prolonged responses in patients who are not candidates for salvage chemotherapy or ASCT. Tafasitamab was developed to increase binding with Fc gamma receptor on immune effector cells and thereby promote antibody dependent-cellular cytotoxicity and phagocytosis.

It has shown single-agent activity with a particularly high duration of response in patients with relapsed/refractory B-cell lymphoma. However, a recent published study has shown a synergystic effect of combining tafasitamab with lenalidomide in patients who were not eligible for ASCT. Of the 81 patients included in the phase 2 study (L-MIND), ORR and CR were documented in 57.5% and 40%, respectively, and the median PFS and OS were 12.1 months and 31.6 months, respectively. After a follow-up of 35 months, the median estimated duration of response was as high as 43.9 months. In addition, tafasitamab in combination with lenalidomide has proven to be more effective as second-line treatment in comparison with third-line treatment or beyond. Double-hit and primary refractory patients were excluded from the study [10, 11].

Tafasitamab should be given in combination with lenalidomide for a maximum of 12 cycles. After completing 12 months of combination therapy, tafasitamab can then be continued as monotherapy until disease progression or unacceptable toxicity. The treatment with tafasitamab and lenalidomide is associated with serious cytopenias in most patients; cytopenias are likely related to lenalidomide use.

Based on the result of the L-MIND study, tafasitamab in conjunction with lenalidomide was approved for the treatment of adult patients with relapsed/refractory transplant ineligible DLBCL.

Bispecific antibodies

Bispecific antibodies (BAs) are engineered proteins that can simultaneously recognize two different antigens. Several CD3/CD20 BAs are currently under development to treat DLBCL.

One of them, glofitamab, is a novel CD20/CD3 T-cell engaging bispecific antibody designed to target CD20 on the surface of B-cells and CD3 on the surface of T-cells. Glofitamab is being investigated in several clinical trials [12].

Data from the phase 2 study demonstrated that glofitamab induces durable CR in patients with heavily pre-treated and refractory DLBCL. After a median follow-up of 12,6 months, 51.6% of patients achieved ORR and 39.4% achieved CR. The majority (77.6%) of complete responses were durable. Cytokine release syndrome was the most common adverse event occurring in 63% of patients [13].

CAR T-cell therapy

In recent years, T-cell genetically modified with chimeric antigen receptor (CARs) has become a therapeutic option that is showing a lot of promise in treatment of relapse/refractory DLBCL. The majority of currently developed CAR T-cells for B-cell lymphoma utilize CD19 as a therapeutic target.

In 2017, data from pivotal ZUMA-1 trial evaluating the 2nd generation CD19 CAR T-cell therapy axicabtagene ciloleucel (axi-cel) proved its exceptional ability to induce complete and persistent clinical responses in chemotherapy-resistant lymphoma patients [14, 15]. Since then, there were two other pivotal trials evaluating 2nd generation CD19 CAR T-cell products for aggressive B-cell lymphoma, including tisagenlekleucel (tisa-cel) and lisocaptagene maraleucel (liso-cel) [16, 17].

Overall, the clinical outcomes are similar amongst the three CAR T-cell products with initial ORR reported between 52-82% and CR rates of 40-54% [14, 16, 17].

In patients with DLBCL, the most recent 5-year results from the JULIET study (tiso-cel) with an average follow-up of 60,7 months indicated an OR rate of 58 percent, a CR rate of 46 percent, and an average DoR of 61,4 months [18].

CAR T-cell therapies are also related to potentially fatal toxicities, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Severe or life-threatening CRS occurs in up to 13% of patients, and up to two thirds of patients treated with axi-cel developed neurologic toxicity [14]. Analysis of clinical data showed that high tumor burden, high T-cell doses and high peak of T-cell expansion can increase the incidence of CRS.

Due to the time necessary for apheresis, production, logistics, and cost, this procedure is inaccessible to many patients despite its effectiveness. Patients are at risk of succumbing to their illness during this time, especially in the absence of an effective bridging treatment.

Based on these recent studies, CAR T-cell therapy appears to result in long-term remission for 30-40% of relapsed illness patients. However, CD19 downregulation represents a common mechanism of relapse after initial responses to CD19 CAR T-cell therapy.

Currently, three different CD19 CAR T-cell therapies are approved for treating adult patients with relapse/refractory large B-cell lymphoma after two or more lines of systemic therapy. Recent studies have been comparing the efficacy of CD19 CAR T-cell therapy with ASCT in patients with early first relapse or primary refractory DLBCL. The conclusion of these studies showed that axi-cel and liso-cel are superior choices for disease control when comparing with ASCT, while tisa-cel was not [19–21].

Selinexor

Selinexor is an orally available selective inhibitor of XPO1-mediated nuclear export (SINE). The mechanism of action appears to be inhibition of nucleocytpolasmic shuttling proteins that functionally inactivate p53 and other tumor suppresor proteins. Selinexor was associated with an objective response in approximately one quarter of highly-selected patients, but it is associated with significant toxicity.

In a multicenter study, 127 patients with extensive pretreatment were administered Selinexor at a fixed dose of 60 mg until disease progression or intolerable toxicity. The overall response rate was 28% and CR rate was 12%. The average duration of response was 9.3 months for all responders and 23.0 months for patients in CR. The most often reported adverse effects in this study were cytopenia and fatigue [22].

Conclusion

In recent years, there has been progress in the treatment of patients with relapsed/refractory Diffuse Large B-cell lymphoma thanks to several new treatment options being approved or pending approval.

In patients with relapsed or refractory lymphoma the choice of therapy depends on comorbidities, previous therapies, toxicity, and availability of therapy. For now, the high dose therapy and autologous stem cell transplantation remains the gold standard of care for chemotherapy sensitive transplant eligible patients. If the patients are transplant ineligible, polatuzumab vedotin in combination with bendamustine and rituximab is the favorable option. The promising response rates and the durability of the response make pola-BR a potential bridging therapy to other approaches, such as stem cell transplantation or Chimeric antigen receptor T-cell therapy in diffuse large B-cell lymphoma.

Recently, chimeric antigen receptor T-cell therapy has demonstrated impressive results and durable responses in multiple treatment-refractory diffuse large B-cell lymphoma, but it is unavailable to many patients with relapsed/refractory diffuse large B-cell lymphoma. In patients who are not candidates for autologous stem cell transplantation or chimeric antigen receptor T-cell therapy, tafasitamab in combination with lenalidomide is an effective treatment option, but treatment requires frequent outpatient infusions and is associated with serious cytopenias. For heavily pretreated diffuse large B-cell lymphoma patients, loncastuximab can be used as a monother-

apy, even after autologous stem cell transplantation or chimeric antigen receptor T-cell therapy failure. If palliation is the intention, the drug of choice would be the oral agent selinexor.

To conclude, novel agents are changing treatment strategies in relapsed/refractory diffuse large B-cell lymphoma. Immunotherapies including antibody-drug conjugates, other monoclonal antibodies and chimeric antigen receptor T-cell therapy have increased the options a clinician has when treating diffuse large B-cell lymphoma patients.

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GREY ZONE LYMPHOMA
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GREY ZONE LYMPHOMA – DIAGNOSTIC AND THERAPEUTIC CHALLENGE

LIMFOM SIVE ZONE – DIJAGNOSTIČKI I TERAPIJSKI IZAZOV

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Summary

Introduction. “Grey zone Lymphoma’’ is associated with various entities. The last published classification of lymphoproliferative neoplasms includes mediastinal grey zone lymphoma. Precise diagnostic criteria are insufficient and establishing a diagnosis is as complex as deciding on treatment options. In this article pathologist and hematologist discuss issues on this topic through case presentation and literature review. **Clinical characteristics.** It presents in the younger population usually with a mediastinal mass, sometimes large, with compressive symptoms. **Pathologic characteristics.** Some cases can’t be classified neither as Hodgkin nor Primary mediastinal B-cell lymphoma. Morphology resembles Hodgkin, but with a positive immunophenotype for primary mediastinal or diffuse large B-cell lymphoma, and vice versa. **Case report.** We presented a case of a 33-year-old male with cervical lymphadenomegaly, B symptoms and clinical deterioration during the diagnostic period. After the first biopsy, differential diagnosis was Epstein-Barr virus-associated lymphoproliferative disorder or classical Hodgkin lymphoma. The second biopsy confirms Epstein-Barr virus-positive diffuse large B-cell lymphoma. **The World Health Organization Classification of lymphoproliferative neoplasms – clinical perspective.** The term “grey zone lymphoma” is associated with overlapping diagnosis or uncertainty in diagnosis in more clinical settings than the ones provided in the 5th World Health Organization Classification. **Discussion.** For now, chemotherapeutic regimen (rituximab-cyclophos phamide, doxorubicin, vincristine, prednisone) stays the standard first line therapy for diffuse large B-cell lymphoma regardless of the Epstein-Barr virus status. Mediastinal grey zone lymphoma treatment varies: chemotherapeutic regimen (rituximab-cyclophos phamide, doxorubicin, vincristine, prednisone) was linked with better outcomes than chemotherapeutic regimen (adriablastin, bleomycin, vinblastine, dacarbazine) +/-R, but for some patients chemotherapeutic regimen (dose adjusted-etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) might be beneficial. **Conclusion.** Grey zone lymphoma is a rare hematologic malignancy that needs extensive sampling for correct diagnosis and is still subject to inter-observer variability.

Key words: Lymphoproliferative Disorders; Lymphoma B-Cell; Classification; Composite Lymphoma; Diagnosis, Differential; Therapeutics; Hodgkin Disease; Lymphoma, Non-Hodgkin; Signs and Symptoms; Mediastinal Neoplasms

Sažetak

Uvod. Limfomi sive zone su povezivani s različitim entitetima. Poslednja klasifikacija limfoproliferativnih neoplazmi uključuje medijastinalni limfom sive zone. Nedostaju precizni dijagnostički kriterijumi tako da je kompleksno i postavljanje dijagnoze i odluka o terapiji. U ovom članku, patolog i hematolog diskutuju o dilemama na ovu temi kroz prikaz slučaja i pregled literature. **Kliničke osobine.** Obično se prezentuje u mlađoj populaciji medijastinalnom masom, ponekad velikom, uz kompresivne tegobe. **Patološke osobine.** Neki slučajevi se ne uklapaju ni u dijagnozu Hočkinovog, ni primarnog medijastinalnog B-limfoma. Izgled ćelija podseća na Hočkinov limfom, ali ima imunofenotip bliži primarnom medijastinalnom ili krupnoćelijskom B-limfomu i obrnuto. **Prikaz slučaja.** Prezentujemo slučaj 33-godine starog muškarca sa cervikalnom limfadenomegalijom, prisutnim B simptomima i kliničkim pogoršanjem tokom perioda dijagnostike. Nakon prve biopsije diferencijalna dijagnoza je uključivala sa Epštajn-Bar virusom povezanu limfoproliferativnu bolest i klasičan Hočkinov limfom. Drugom biopsijom postavljena je dijagnoza Epštajn-Bar virus pozitivnog krupnoćelijskog B-limfoma. **Klasifikacija Svetske zdravstvene organizacije limfoproliferativnih neoplazmi – perspektiva kliničara.** Termin „siva zona limfoma“ podseća na preklapajuće dijagnoze ili nesigurnost u dijagnozu u više kliničkih situacija u odnosu na one s kojima je povezan u 5. Klasifikaciji Svetske zdravstvene organizacije. **Diskusija.** Za sada je R-CHOP standardna prva terapijska linija u lečenju difuznog krupnoćelijskog B-limfoma, nezavisno od Epštajn-Bar virus-statusa. Lečenje medijastinalnog limfoma sive zone se razlikuje: R-CHOP se povezuje s boljim ishodom u odnosu na ABVD+/-R, a kod nekih slučajeva DA-EPOCH-R može imati prednost. **Zaključak.** Limfom sive zone je redak hematološki malignitet koji zahteva ekstenzivno uzorkovanje za tačnu dijagnozu uz i dalje veliku razliku u interpretaciji nalaza.

Ključne reči: limfoproliferativni poremećaji; B-ćelijski limfom; klasifikacija; kompozitni limfom; diferencijalna dijagnoza; terapija; Hočkinova bolest; non-Hočkinov limfom; znaci i simptomi; medijastinalna neoplazma

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Abbreviations

ABVD	– chemotherapeutic regimen (adriablastin, bleomycin, vinblastine, dacarbazine)
CD	– cluster of differentiation
CHL	– classical Hodgkin lymphoma
CRP	– C reactive protein
CT	– computed tomography
DA-EPOCH	– chemotherapeutic regimen (dose adjusted-etoposide, prednisone, vincristine, cyclophosphamide, doxo rubicin)
DLBCL	– diffuse large B-cell lymphoma
EBER	– Epstein-Barr virus (EBV) (EBV-encoded small RNA) in situ hybridization
EBV	– Epstein-Barr virus
ESR	– erythrocyte sedimentation rate
GZL	– Grey zone lymphoma
LDH	– lactate dehydrogenase
LPD	– lymphoproliferative disorder
LPN	– Lymphoproliferative Neoplasm
MGZL	– Mediastinal grey zone lymphoma
MRCP	– Magnetic resonance cholangiopancreatography
NLPHL	– Nodular lymphocyte predominant Hodgkin lymphoma
NOS	– not otherwise specified
NSCHL	– nodular sclerosis classical Hodgkin lymphoma
PMBL	– primary mediastinal large B-cell lymphoma
PD-1	– programmed cell death protein-1
PD-L-1	– programmed cell death ligand-1
R-CHOP	– chemotherapeutic regimen (rituximab-cyclophos phamide, doxorubicin, vincristine, prednisone)
SEER	– The Surveillance, Epidemiology, and End Results
WHO	– World Health Organization

Introduction

Grey zone lymphoma (GZL) is an unclassifiable B-cell lymphoma sharing features with diffuse large B-cell lymphoma (DLBCL), namely primary mediastinal large B-cell lymphoma (PMBL), and classical Hodgkin lymphoma (CHL), especially nodular sclerosis subtype (NSCHL) [1]. According to the upcoming 5th edition of the World Health Organization (WHO) Classification of Hematolymphoid Tumors, the term "Mediastinal grey zone lymphoma (MGZL)" replaced the previous entry "B-cell lymphoma, unclassifiable with features intermediate between DLBCL and classic Hodgkin lymphoma" [2]. Data from the SEER Registry of The National Cancer Institute show that before 2010 the term for this code was composite Hodgkin and non-Hodgkin lymphoma. In the 2008 WHO Classification, the term used to describe this same disease was B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma. The change in terminology came from findings showing that the non-Hodgkin proliferation part of this lymphoma is of the B-cell immunophenotype [3]. Also, alternative names include Composite Hodgkin and non-Hodgkin lymphoma, Composite lymphoma, Hodgkin-like anaplastic large cell lymphoma, Large B-cell lymphoma with Hodgkin features, Mediastinal grey-zone lymphoma - MGZL [3]. It affects mainly young males aged 20-40 years. Due to the primary mediastinal occurrence, they are also termed mediastinal GZL (MGZL). However, rare cases in the peripheral lymph nodes as the primary site are referred to as non-MGZLs. The term "grey zone lymphoma" should be restricted primarily to those cases with mediastinal localization. However, in time, the concept became more broadly applied, and GZL was used for cases that also overlap with other B-NHL types. The term GZL was first recognized in 1998 as a possible clinicopathological entity at a workshop for Hodgkin's disease. It was used to help classify a subset of mediastinal B-cell lymphomas with features of overlapping Hodgkin lymphoma [4]. Since GZL covers a variety of morphologies and immunophenotypes, it lacks precise diagnostic criteria. Despite new diagnostic capabilities and achievements, establishing a diagnosis is complex, as are clinical decisions about treatment. First-line therapy differs between CHL and PMBL or DLBCL. In this article, a pathologist and a hematologist discuss issues on this topic from different perspectives, through case presentation and literature review.

Clinical characteristics

It usually presents in a younger population within the third decade, with a large mediastinal mass, sometimes with supraclavicular lymph node involvement and additional symptoms like dyspnea or superior vena cava syndrome caused by compression [1]. Non-mediastinal GZL, typically presenting in older age, comes with supraclavicular and cervical lymphadenopathy, while peripheral and intra-abdominal are less common [5–7]. There may be direct spreading to the lungs, liver, spleen, and, rarely, bone marrow. Non-lymphoid organs are rarely involved, unlike in PMBL.

Morphology and immunophenotype characteristics

Since the anterior mediastinum is a common site for both NSCHL and PMBL, some cases do not fit these diagnoses because of features intermediate between DLBCL and CHL, but show transitional features - unclassifiable B-cell lymphoma. NSCHL is characterized by cellular nodules consisting of scant mononuclear Hodgkin and multinucleated Reed-Sternberg (HRS) cells of the lacunar variant with an inflammatory background of reactive lymphocytes, eosinophils, histiocytes and plasma cells surrounded by collagen bundles creating nodular formations [8, 9]. The classical immunophenotype shows an expression of CD30 and CD15, MUM1, weak PAX5, no or only weak heterogeneous expression of CD20, CD79a, BOB1, and OCT2. The strong positivity of a single B-cell marker like CD20 should not automatically define the GZL category. EBV positivity is observed in a minority of cases (10-20%) [1, 10]. PMBL is a rare, specific subtype of large B-cell lymphoma that usually affects young females, with a large mediastinal mass and frequently compressive symptoms at presentation. Although it is more aggressive than CHL, there is a rare bone marrow or nodal involvement, and the

prognosis is better than MGZL. It originates from thymic B-cells with a diffuse proliferation of medium to large cells with abundant, pale cytoplasm and round to ovoid nuclei. It is commonly associated with compartmentalizing and alveolar fibrosis, rather than nodule formation as in NSCHL. Lymphoma cells can have pleomorphic and/or multilobulated nuclei that may resemble lacunar HRS cells [11]. There is immunoexpression of pan-B cell markers like CD20, CD79a, PAX5, OCT2, and BOB1 with a frequent expression of CD23 (~70%) and MAL, a highly specific marker for PMBL. Also, CD30 is positive in most cases (>80%), and CD15 is generally negative. Markers MUM1 and BCL6 are frequently expressed, while CD10 is rarely positive. EBV positivity is an exclusionary marker for PMBL [12]. Unclassifiable B-cell lymphoma was presented in 1998 as a new category [4] and further detailed in 2005 [13]. The 2008 WHO classification incorporated a different entity as unclassifiable B-cell lymphoma with features between DLBCL and CHL. Due to the variation of the morphological spectrum of GZL, the most important feature required for considering the GZL diagnosis is the high tumor cell population in most of the tumor areas [1]. It can more closely resemble PMBL/DLBCL with sheets of large centroblastic cells with clear cytoplasm, while other cases may exhibit marked pleomorphism [10]. Also, it can resemble CHL more closely, showing typical lacunar and HRS cells with a background of mixed inflammatory infiltrate [13]. Unlike many CHLs, tumor cells are abundant, frequently in confluent sheets, which is a significant diagnostic detail, and nuclei are different in size and shape with less acidophilic nucleoli than traditional HRS. The next important feature of GZL is the lack of nodular pattern with the presence of only focal fibrosis, unlike in CHL [10]. Necrotic areas with no neutrophil infiltrate can be observed in contrast to CHL, where only small focuses of necrosis with neutrophils can be found [1]. Immunohistochemically there is an overlap of both CHL and PMBL/DLBCL, but cases morphologically resembling NSCHL usually show CD20, CD79a, PAX5, OCT2, and BOB1 expression and CD15 negativity (CD30 and CD15 are variable expressed), while cases that are morphologically closer to PMBL/DLBCL exhibit loss of B-cell markers with strong positivity of CD30 and frequently CD15 or demonstrating strong CD20, CD30, and CD15 so-called "group 3" cases described by the Lymphoma Study Association [7]. Most MGZL have an expression of B-cell markers such as CD20, CD79a, PAX5, OCT2, and BOB1, and rare cases show EBV positivity, so it remains controversial whether EBV-positive cases should be called GZL or EBV-related entities [14, 15]. A recommended immunohistochemical panel for the diagnosis of GZL includes CD20, CD79a, PAX5, CD3, CD30, CD15, MUM1, and Epstein-Barr virus (EBV) (EBV-encoded small RNA) in situ hybridization (EBER) [10]. In relapse cases, PD-L1 testing can be useful for therapeutic purposes. Many genetic studies and investigations showed that NSCHL, PMBL, and MGZL are biologically related entities [14, 16]. Despite the loss of B-cell markers of HRS cells, they have B-cell genotypes and carry somatically mutated and clonally rearranged IGH genes. Also, those three entities share amplifications of 2p16-containing the REL oncogene, and 9p24- containing JAK2 and programmed-death ligand genes PD1L1 and PD1L2. A recent study showed that MGZL exhibits SOCS1, B2M, TNFAIP3, GNA13, STAT6, and NFKBIA as the most frequent gene mutations with a lack of BCL2 and BCL6 translocations. In contrast to DLBCL, the most frequent mutations CARD11, MYD88, CD79B, or EZH2 were not registered in MGZL. In conclusion, mediastinal NSCHL, PMBL, and MGZL probably share thymic B-cell origin and represent a clinical, biological, and genetic continuum with distinct architectural features [9].

Case report

A 33-year-old male came to a hematologist complaining about night sweating, fever, and weight loss. Cervical lymph node enlargement had been noticed for six months, but worsened a couple of weeks before the first visit to the hematologist. He had increased ESR, the concentration of LDH, CRP, beta2microglobulin, ferritin, and low iron concentration. A biopsy of a cervical lymph node was performed, and the morphology and immunophenotype of the tumor cells were consistent with EBV-positive lymphoproliferative disorder (LPD). The diffuse growth pattern of predominantly medium atypical lymphoid cells with many large cells of immunoblast morphology type was described, among some expressed B-cell markers and EBER positivity and other expressed T-cell markers. Also, some cells of classical HRS cell morphology were observed with partly CD30 expression and CD15 negativity. In the background, there were lymphocytes, histiocytes, eosinophils, and plasma cells with no necrosis. After revision of the same blocks with additional immunohistochemical markers and clonality of T- and B-cell population, the report stated that it was an EBV-related LPD, but that could also be CHL as a differential, so the clinicopathological correlation was warranted or another biopsy.

Meanwhile, our patient became anemic with the progression of symptoms and lymphadenopathy up to 8 cm in diameter, spleen enlargement, focal infiltration, and no bone marrow infiltration. We decided to do a biopsy of another cervical lymph node and started with corticosteroid therapy, which resulted in clinical improvement.

Having performed appropriate morphological and immunohistochemical analyses, a new pathohistological report confirmed EBV-positive DLBCL, NOS. The atypical lymphoid cells were of medium and large size with single sparse HRS-like cells. Immunohistochemically, they were positive for CD20, PAX5, MUM1, EBV, and CD30 focally

with proliferation index Ki67 about 30-40%. The patient was treated with R-CHOP, and after the third cycle, a partial response was achieved. Therapy was continued, but after the fifth course, he came down with abdominal pain and fever, focal liver infiltration, and abdominal and mediastinal lymphadenopathy seen on CT scan and MRCP consistent with progression of the disease.

Salvage therapy with R-DHAP regimen was given, but in a period of post-therapy aplasia, the patient tested positive for SARS-CoV 2. After four days, chest radiography showed massive pneumonia. During hospitalization, he was treated with antimicrobial and supportive therapy. A follow-up CT scan revealed the resolution of hepatic lesions and abdominal lymphadenopathy, but with respiratory deterioration and multiorgan failure, the patient passed away one month later.

The WHO Classification of LPN - clinical point of view

The term "grey zone lymphoma" to clinicians without a certain amount of pathologic knowledge is a bit confusing. It is associated with an overlapping diagnosis or uncertainty in diagnosis in more clinical settings then the ones provided in the 5th WHO Classification. The term has been used for hybrid features between NLPHL and T-cell/histiocyte rich DLBCL, or EBV positive cHL and EBV positive DLBCL [17, 18].

In the International Consensus Classification of Mature Lymphoid Neoplasms authors stated that EBV-positive polymorphic B-cell lymphoproliferative disorder, NOS is a term used for EBV-positive B-cell proliferation with or without known immunodeficiency that cannot be more precisely categorized. The term should be reserved for cases with altered lymph node architecture and a polymorphic infiltrate that do not fulfill the criteria for the diagnosis of lymphoma or where there is uncertainty due to a small size or low-quality biopsy [19–21].

On the other hand, EBV-positive DLBCL could occur in immune deficiency and dysregulation, which is another group in the classification. The first-line therapy in DLBCL, according to guidelines, regardless of EBV status, is R-CHOP. More and more patients are on various immunosuppressive or immunomodulatory treatments for different diseases. It seems reasonable to expect more lymphoid proliferations and lymphomas associated with immune deficiency and dysregulation. We would probably need and hopefully get additional treatment recommendations in the near future.

Discussion

In the presented case, the first result suggested EBV-positive LPD. After additional staining for MUM 1 and EBER, the response was that it was EBV-positive polymorphic LPD or CHL. However, the number of RS cells and variants was small and clinical correlation was warranted. Meanwhile, the state of our patient deteriorated, and dilemmas regarding therapy arose: based on literature data that EBV-related LPD could be treated only with immunotherapy with rituximab [22–25] or treat him like CHL with ABVD because from the clinician's point of view it seemed like a malignant lymphoproliferation. On the other hand, there was uncertainty about the diagnosis, and we decided to perform the second biopsy and start with prednisolone therapy. Clinical improvement was seen, and the final diagnosis was EBV-positive DLBCL, NOS. In previous reports, this type of DLBCL was linked to the elderly and had an inferior outcome on chemotherapy compared with EBV-negative DLBCL [26–30]. Later, it was also reported in the younger population, changing names in the 4th WHO Classification [31]. Immunochemotherapy with R-CHOP improved outcomes in this disease, but there are many open questions. Antiviral drugs do not eradicate EBV from transformed B-cells because EBV is in a latent phase in affected B cells. However, there are suggestions about a possible role of novel therapy like histone deacetylase inhibitors or proteasome inhibitors in the induction of the lithic phase, which could lead to effective exposure to antiviral drugs [30, 32]. Quan et al. showed that PD-1 blockade was more effective in PD-L1-positive EBV-positive DLBCL than in PD-L1-positive EBV-negative DLBCL [33]. While waiting for ongoing study results, R-CHOP stays the standard first-line therapy. MGZL treatment varies: R-CHOP was linked with better outcomes than ABVD, but DA-EPOCH-R might be beneficial for some patients.

Conclusion

Diagnosis and classification of grey zone lymphoma remain challenging for both pathologists and hematologists. Grey zone lymphoma is a rare hematologic malignancy that needs extensive sampling for correct diagnosis and is still subject to inter-observer variability. Currently, morphologic and immunophenotypic evaluation with other diagnostic possibilities is required to reach the correct final diagnosis, which is vital for optimal treatment.

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"DOUBLE EXPRESSOR" DIFFUSE LARGE B-CELL LYMPHOMA – A CASE REPORT AND LITERATURE REVIEW

DIFUZNI B-KRUPNOĆELIJSKI LIMFOM SA DVOSTRUKOM EKSPRESIJOM – PRIKAZ SLUČAJA I PREGLED LITERATURE

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Summary
Diffuse large B-cell lymphoma, not otherwise specified, is the most common type of non-Hodgkin lymphoma worldwide, accounting for 30–40% of all lymphomas. It represents a collection of morphologically, genetically and clinically different diseases. Therefore, it can be subdivided into morphological variants, phenotypic subtypes, and molecular or genetic categories. More recently, diffuse large B-cell lymphoma has witnessed advances in molecular profiling and treatment of patients with refractory and relapsed disease. The optimal management requires integrated morphological and immunophenotypic analysis of cell and tissue, along with chromosome and molecular analyses. Double-expressor lymphoma, defined as overexpression of MYC and BCL2 proteins not related to underlying chromosomal rearrangements, accounts for 20% to 30% of Diffuse large B-cell lymphoma cases. In the latest, 5th edition of the World Health Organization Classification of Hematolymphoid Tumors-lymphoid neoplasms, double-expressor lymphoma is not defined as an independent entity, but it has been proven to be a marker for poor outcome in diffuse large B-cell lymphoma. However, the degree of adverse prognosis is lesser than in double-hit lymphomas. Although double-expressor lymphoma feature is confirmed as adverse prognostic marker for diffuse large B-cell lymphoma patients, currently no sufficient data is available to support treatment intensification over standard rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone regimen. Well-designed randomized clinical trials are mandatory in order to properly respond to this substantial clinical dispute.
Key words: Lymphoma, Large B-Cell, Diffuse; Lymphoma, Non-Hodgkin; Classification; Prognosis; Therapeutics; Algorithms

Sažetak
Difuzni B-krupnoćelijski limfom, bez daljeg određenja, najčešći je tip ne-Hočkinovog limfoma širom sveta i čini 30−40% svih limfoma. Predstavlja skup morfološki, genetski i klinički različitih bolesti. Stoga se može podeliti na morfološke varijante, fenotipske podtipove i molekularne ili genetske kategorije. U skorije vreme evidentan je napredak u molekularnom profilisanju difuznog B-krupnoćelijskog limfoma i lečenju pacijenata sa refraktarnom i relapsnom bolešću. Preduslov optimalnog tretmana je integrisana morfološka i imunofenotipska analiza ćelija i tkiva, uz hromozomsku i molekularnu analizu. *Double-expressor* limfom, definisan kao limfom sa prekomernom ekspresijom MYC i BCL2 proteina bez prisustva rearanžmana MYC/BCL2 gena, čini oko 20−30% svih difuznih B-krupnoćelijskih limfoma. U poslednjem 5. izdanju Klasifikacije hematolimfoidnih tumora – limfoidnih neoplazmi Svetske zdravstvene organizacije, *double-expressor* limfom nije definisan kao nezavisni entitet, ali je dokazano da je negativan prognostički marker za difuzni B-krupnoćelijski limfom. Međutim, stepen nepovoljne prognoze je manji nego u *Double-hit* limfomima. Iako je *double-expressor* limfom potvrđen kao negativan prognostički marker za pacijente sa difuznim B-krupnoćelijskim limfomom, trenutno nema dovoljno podataka koji idu u prilog intenziviranju terapije u odnosu na standardno lečenje protokolom *rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone.* Dobro dizajnirana randomizovana klinička studija je neophodna u cilju dobijanja adekvatnog nivoa dokaza za rešavanje ovog značajnog kliničkog problema.
Ključne reči: difuzni krupnoćelijski limfom; non-Hočkinov limfom; klasifikacija; prognoza; terapija; algoritam

Introduction

Diffuse large B-cell lymphoma, not otherwise specified (DLBCL-NOS) (hereinafter referred to as "DLBCL") is the most common type of non-Hodgkin lymphoma worldwide, accounting for 30–40% of all lymphomas. It is not a single disease, but a collection of morphologically, genetically and clinically different diseases. Therefore, it can be subdivided into morphological variants, phenotypic subtypes, and molecular or genetic categories. More recently, DLBCL has witnessed advances in molecular profiling and treatment of patients with refractory and relapsed disease.

The objective of this study was to point to recent advances in diagnostic stratification of DLBCL, as well as clinical implication and treatment of some more aggressive subtypes of DLBCL.

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Abbreviations

DLBCL-NOS – diffuse large B-cell lymphoma, not otherwise specified
MRI – magnetic resonance imaging
LS – lumbo-sacral
PH – pathohistological
IHC – immunohistochemical
DEL – "double expressor" diffuse large B-cell lymphoma
FISH – fluorescent in situ hybridization
CT – computerized tomography
SE – erythrocyte sedimentation rate
CRP – C-reactive protein
LDH – lactate dehydrogenase
ECOG PS – Eastern Cooperative Oncology Group performance status
CS – clinical stage
R-IPI – Revised International Prognostic Index
CNS-IPI – Central Nervous System International Prognostic Index
R-CHOP – rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone
HD-MTX – high dose methotrexate
PET/CT – positron emission tomography/CT scan
COO – cell of origin
GEP – gene expression profiling
ICC – International Consensus Classification
DHL – double-hit lymphomas
HGBCL – high-grade B-cell lymphoma
CR – complete remission
OS – overall survival
PFS – progression-free survival
(DA) R-EPOCH – (dose adjusted) rituximab, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone
NCCN – National Comprehensive Caner Network

Case report

A 46-year-old male patient complained of pain in his left leg at the beginning of August 2019. According to personal medical history, the patient was without chronic diseases and prior major surgical interventions. Physical therapy was initiated in a private health institution; however, due to the persistence of the complaints, the patient underwent magnetic resonance imaging (MRI) of the lumbo-sacral (LS) spine (21.08.2019.), which showed a tumor mass of the spinal canal from L4 to L5 with a diameter of 61 x 50 x 20 mm, with compression on the left radix of L5. After adequate preoperative preparation, the patient was operated on 05.09.2019., when L4/L5 hemilaminectomy, extradural tumor reduction and spinal canal decompression were performed. Pathohistological (PH) and immunohistochemical (IHC) findings were the following: IHC tumor cells are: PAX-5+, CD20+, CD79α+, CD3-, CD5-, bcl-2+, bcl-6+, CD10-, MUM-1-, c-myc+, CD23-, Cyclin D1-, CD15-, CD30+, CD138-, ALK-1-, EMA-, EBV-LMP-, Ki-67+ in about 50% of tumor cells; conclusion: morphological and IHC findings most closely correspond to "double expressor" diffuse large B-cell lymphoma (DEL). The rearrangements of the c-myc, bcl-2 and bcl-6 genes were ruled out using the fluorescent in situ hybridization (FISH) method. As part of disease staging procedures, a bone marrow biopsy was performed that revealed lymphoma cells infiltration (around 20%). A control postoperative computerized tomography (CT) scan of LS spine was performed (30.10.2019.), which showed the presence of a tumor mass at the L4/L5 level with a consequent bone tissue defect, while the dimensions of the tumor mass were not precisely defined. CT scan of the neck, chest, abdomen and pelvis (31.10.2019.): on the lateral crus of the right adrenal gland, a nodule with a diameter of 16 x 13 mm. Other findings were normal. Virological analyses (HCV, HBsAg, HIV) negative. Echocardiography showed normal findings, with left ventricular ejection fraction around 60%. In pre-treatment laboratory analyses, the complete blood count with leukocyte formula was within reference values, as well as the findings of biochemical analyses (erythrocyte sedimentation rate (SE) 2 mm/h, C-reactive protein (CRP) 0.7 mg/l, lactate dehydrogenase (LDH) 166 U/L, beta 2 microglobulin 2.39 mg/l). In the physical examination, peripheral lymphadenopathy and hepatosplenomegaly were absent, ECOG PS (Eastern Cooperative Oncology Group performance status) was 1. Diagnostic lumbar puncture was done, and cytologic and flow-cytometry analysis of the cerebrospinal fluid was negative for lymphoma cells presence. After the completion of all diagnostic procedures, the patient was staged as IV A clinical stage (CS) (Ann Arbor), R-IPI 2 (Revised International Prognostic Index), CNS IPI 2 (Central Nervous System International Prognostic Index). Because of the specific extranodal localization of the lymphoma, the patient was considered a candidate for CNS prophylaxis. The chemoimmunotherapy with R-CHOP protocol (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) was initiated coupled with intercalated high dose methotrexate (HD-MTX) CNS prophylaxis. Overall, 6 cycles of R-CHOP and 3 cycles of intercalated HD-MTX were administered by March 2020., control CT scan showed significant, but incomplete, reduction of the epidural tumorous mass. Adjuvant radiotherapy of L3-S2 region with 45 Gray was applied, while control MRI of LS spine revealed the tumor mass in significant, but incomplete, regression. Furthermore, whole body positron emission tomography/CT scan (PET/CT) found no disease activity. The therapy resulted in remission, which is maintained for two years.

Recent advances in diagnostic stratification, clinical implication and treatment of DLBCL

Optimal management requires integrated morphological and immunophenotypic analysis of cell and tissue, together with chromosome and molecular analyses. The standardization of these techniques has improved their reproducibility and consequently their inclusion in evolving classifications of mature lymphoid malignancies [1]. There are some recent advances in diagnostic stratification of DLBCL.

Morphologic variants

DLBCL is cytologically heterogeneous, with three common morphologic variants recognized in the WHO classification [1], but it is not required to report them. They are the centroblastic, immunoblastic, and anaplastic variant. Some studies have suggested a worse outcome for patients with immunoblastic variant [2], which is more frequently associated with a non-GCB phenotype (94% vs. 6%) [3].

Immunophenotypic subtypes

The Hans algorithm uses three markers to distinguish the GCB from the non-GCB subtype: CD10, BCL6, and IRF4/MUM1 are each considered positive if ≥ 30% of the tumor cells stain positively [4].

– Germinal center B-cell type (GCB): CD10+ or CD10−/BCL6+/MUM1− (Hans)

– Non-germinal center B-cell (non-GCB): CD10−/BCL6− or CD10−/BCL6+/MUM1+ (Hans)

– CD5 positive DLBCLs usually constitute de novo DLBCL (the neoplastic cells express CD5 in 5-10% of the cases) [5]

– DEL: co-expression of the MYC and BCL2 proteins (the immunohistochemical threshold of ≥ 40% for MYC and > 50% for BCL2 is used to define DEL), without underlying genomic rearrangements [1]

Cell of Origin Variants

The cell of origin (COO) classification has been the most significant development in the understanding of DLBCL biology. The gold standard test to identify COO is Gene expression profiling (GEP). GEP divides DLBCL into germinal center B-cell (GCB), activated B-cell (ABC) or unclassifiable (Type 3) types.

According to the recent International Consensus Classification (ICC) [6] the role of morphological variants and phenotypic variants such as "DLBCL, CD5+" and "DLBCL, double expressor (MYC/BCL2)" should be deemphasized. These variants have (weak) adverse prognostic impact and do not reflect true biological subgroups, but rather represent the end results of different biological pathways.(6In addition, several key cytogenetic alterations and the abnormal expression of certain proteins have also been shown to affect treatment response and clinical outcome of patients with DLBCL [7]. Recently, MYC, BCL2 and/or BCL6 rearrangements and protein expression levels were identified as prognostic factors in DLBCL, especially for MYC [8].

Double-hit lymphomas/double expressor lymphomas

In the revised WHO classification [1], the starting point for the classification of aggressive B-cell lymphomas is the pathologic appearance with one of the following morphologic descriptions: blastoid, Burkitt, DLBCL/BL, or DLBCL. The second step is cytogenetic or FISH testing for rearrangements of MYC, BCL2, and BCL6. The International Consensus Classification (ICC) [6] included two categories of high-grade B-cell lymphoma (HGBCL): HGBCL, NOS, and HGBCL-DH, which now comprises two groups: HGBCL with MYC and BCL2 rearrangements (with or without BCL6 rearrangement) (HGBCL-DH-BCL2) and a new provisional entity, HBGBL with MYC and BCL6 rearrangements (HGBCL-DH-BCL6).

MYC is rearranged in 5–15% of DLBCL and is frequently associated with BCL2 or to a lesser extent, BCL6 translocation. They are more aggressive and have a much poorer prognosis. Up to 20% of patients with double-hit lymphomas (DHL) do not demonstrate overexpression of MYC and BCL2 at a protein level, and it appears that this population of patients may have improved outcomes in comparison with those with DHL with concurrent dual protein expression [8, 9]. Although the majority of all DHL cases will have co-expression of the respective proteins, the reverse is not necessarily true. As noted previously, patients with co-expression of MYC and BCL2 proteins (without underlying genomic rearrangements) are now considered to have double expressor lymphomas **(**DEL). Importantly, DEL is considered an adverse prognostic indicator but not a separate diagnostic entity in DLBCL [1]. MYC and BCL2 over-expression is likely attributable to gene amplification and post-translational processes in the absence of chromosomal translocations [10]. In terms of biology, the entity DHL and the prognostic group DEL overlap with the concept of COO. Because immunohistochemistry is more commonly used to assess COO than gene expression profiling, the terms have evolved to GCB and non-GCB DLBCL (which includes both ABC DLBCL and other non-GCB samples) [11]. With respect to COO, 80% to 90% of DHL cases occur with GCB DLBCL [12]. Similarly, DEL appears to be highly correlated with the non-GCB subset, with retrospective studies demonstrating that 63% of non-GCB cases harbor dual-protein expression versus 37% of GCB cases [13].

FISH for MYC, BCL2 and BCL6 gene rearrangements should be tested for patients with expression of MYC and either BCL2 or BCL6 by IHC and a GCB like immunophenotype to identify these double-hit lymphomas. However, to definitively rule out DHL, testing all aggressive lymphoma specimens with FISH would be required.

Those patients with high expression of MYC and BCL2 protein by IHC alone in the absence of gene rearrangements by FISH are labeled as double expressor and they seem to have an intermediate prognosis [14].

Clinical implication of double expressor lymphomas and treatment approach

DLBCL encompasses a heterogeneous group of clinically aggressive lymphoma with various thera-

peutic response on standard chemoimmunotherapy. In spite of the emphasized diversity under DLBCL diagnosis, the standard of care remains the R-CHOP protocol [15]. In the latest, 5th edition of the World Health Organization Classification of Hematolymphoid Tumors-lymphoid neoplasms [16], DEL is not defined as independent entity, but it has been proven to be a marker for poor outcome in DLBCL. However, the degree of adverse prognosis is lesser than in DHL [15]. Moreover, the biology of DEL differs significantly in regard to DHL [17]. It is estimated that between 20-30% or DLBCL have DEL expression profile [8].

Initially, Green et al. [13] in 2012 have found that DEL DLBCL was significantly associated with adverse outcomes, precisely with lower complete remission (CR) rate, shorter overall survival (OS) and shorter progression-free survival (PFS), while Johnson et al. [18], similarly, demonstrated inferior OS and PFS in DEL DLBCL. After these breakthrough findings, it was reported that DEL has a more aggressive clinical course and dismal prognosis [8, 19, 20]. Moreover, DEL is more frequent in ABC subtype, contributing to inferior prognosis of ABC-DLBCL [17]. Inferior and insufficient response to both standard first line therapy [19, 21] and in relapsed/refractory settings [9, 22] have been observed. In addition, DEL phenotype is associated significantly with the female gender, older age, higher Ki-67 [23], elevated LDH level [24], poor performance status, higher IPI score and higher incidence of extranodal sites [25]. Treatment intensification in first line settings is a matter of debate and controversy, raising questions and confusion between clinicians. Considering this as a grey area that is often encountered in daily practice, the therapeutic approach is known to vary between institutions, which is adequately reflected in the publications. Suboptimal response to R-CHOP of DEL DLBCL was observed in the systematic review and meta-analysis by Lu et al. [26], consequently resulting in shorter OS. Further, more publications came to the similar conclusion [13, 17, 18]. Indeed, these data have shifted both scientific and clinical interest towards more aggressive therapeutic regimens, anticipating positive results. In contrast, the results of many studies were not as straightforward as expected. Firstly, the study by MD Anderson Cancer Center in 2016 [27] identified no statistical significant benefit of dose adjusted (DA) R-EPOCH (rituximab, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone) against R-CHOP, but, based on results, they concluded that (DA) R-EPOCH is highly effective in high-risk DLBCL patients. Notwithstanding, a small study from the same year [28] showed a favorable treatment outcome with R-EPOCH in comparison with R-CHOP in DEL DLBCL patients. In the following years, the number of researches that do not favor therapy intensification for patients with DEL [29–31] notably outnumbered those who do favor intensification [32]. Systematic review and meta-analysis by Hwang et al. [33], that included 7054 patients from 41 studies, confirmed that CR rate is significantly lower in newly diagnosed DEL than non-DEL DLBCL patients. However, one of the main limitations of the study was its inability to compare different treatment approaches and CR rates due to insufficient data.

Latest treatment guidelines for DLBCL issued by the National Comprehensive Caner Network (NCCN) [34] emphasized that there is no evidence that an intensified regimen is an adequate treatment option for DEL, for whom R-CHOP remains the standard of care.

Conclusion

Beside double expressor lymphomas feature is confirmed as adverse prognostic marker for diffuse large B-cell lymphoma patients, currently there is no sufficient data to support treatment intensification over standard rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone regimen. Well-designed randomized clinical trials are mandatory in order to properly respond to this substantial clinical dispute.

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NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA WITH T-CELL/HISTIOCYTE - RICH LARGE B-CELL LYMPHOMA PATHOHISTOLOGICAL CHARACTERISTICS AND EXTRANODAL PRESENTATION – CASE REPORT AND LITERATURE REVIEW

HOČKINOV LIMFOM, TIP NODULARNE LIMFOCITNE PREDOMINACIJE SA KARAKTERISTIKAMA T-ĆELIJSKOG/HISTIOCITIMA BOGATOG B-KRUPNOĆELIJSKOG LIMFOMA I EKSTRANODALNOM PREZENTACIJOM – PRIKAZ SLUČAJA I PREGLED LITERATURE

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Summary

Nodular lymphocyte predominant Hodgkin lymphoma appears in 5% of Hodgkin lymphoma. Because of major biological and clinical differences with classical Hodgkin lymphoma and close relationship to T-cell/histiocyte-rich large B-cell lymphoma, lately the term nodular lymphocyte predominant B-cell lymphoma is accepted. The presence of lymphocyte predominant cells with preserved B-cell phenotype and a lack of CD30 is the prerequisite for the diagnosis of nodular lymphocyte predominant Hodgkin lymphoma. Lymphocyte predominant cells are typically embedded in large nodules of B lymphocytes (growth patterns A and B), but variants that are characterized by lymphocyte predominant cells located outside the nodules, a T-cell–rich nodular growth pattern and T-cell–rich or B-cell–rich diffuse growth patterns, respectively, have also been described (growth patterns C, D, E, and F). Variant growth patterns are associated with the recurrence and progression of disease and should be recognized and specified in pathology reports. Broad B-cell immunohistochemical panel, including PAX5, CD79a, Bob.1, and Oct-2 is indicated in these cases to distinguish between nodular lymphocyte predominant Hodgkin lymphoma, classical Hodgkin lymphoma and T-cell/histiocyte-rich large B-cell lymphoma, which have significant differences in clinical behavior and treatment. There are different treatment approaches in patients with nodular lymphocyte predominant Hodgkin lymphoma depending on pathohistological type, clinical presentation and stage of the disease. Treatment may include active surveillance, radiation therapy, immunotherapy or chemotherapy. A multidisciplinary approach is beneficial to optimize the diagnosis and management of patients with nodular lymphocyte predominant Hodgkin lymphoma.

Key words: Hodgkin Disease; B-Lymphocytes; Diagnosis; Morphological and Microscopic Findings; Therapeutics; Treatment Outcome

Sažetak

Hočkinov limfom, tip nodularne limfocitne predominacije predstavlja svega 5% Hodgčkinovih limfoma. Nedavno, prihvaćen je termin nodularni limfocitima predominantni B-ćelijski limfom, zbog značajnih bioloških i kliničkih razlika u odnosu na klasični Hočkinov limfom, kao i povezanosti sa T-ćelijskim/histiocitima bogatim B-krupnoćelijskim limfomom. Prisustvo ćelija limfocitne predominacije sa očuvanim B-ćelijskim fenotipom i nedostatkom CD30 je neophodno za postavljanje dijagnoze Hodgčkinovog limfoma, tipa nodularne limfocitne predominacije. Ćelije limfocitne predominacije su tipično ugrađene u velike noduse B-limfocita (morfološka varijanta A i B). Takođe, opisane su morfološke varijante koje karakterišu ćelije limfocitne predominacije lokalizovane izvan nodusa, T-ćelijama bogata nodularna i B-ćelijama bogata difuzna morfološka varijanta (C, D, E i F). Morfološke varijante su povezane sa relapsom i progresijom bolesti, zbog čega je neophodno da budu jasno naglašene u patohistološkim nalazima. Proširen B-ćelijski imunohistohemijski panel, uključujući PAX5, CD79a, Bob.1 i Oct-2, neophodan je radi postavljanja precizne dijagnoze Hočkinovog limfoma, tipa nodularne limfocitne predominacije, klasičnog Hodgčkinovog limfoma, ili T-ćelijskog/histiocitima bogatog B-krupnoćelijskog limfoma, što je od značaja zbog razlika u kliničkoj prezentaciji i terapijskim modaltetima. Različiti su terapijski pristupi pacijenatima sa Hodgčkinovim limfomom, tipa nodularne limfocitne predominacije u zavisnosti od patohistološkog tipa, kliničke slike i stadijuma bolesti. Terapija obuhvata aktivno praćenje, zračnu terapiju, imunoterapiju i hemioterapiju. Multidisciplinarni pristup kod bolesnika sa Hodgčkinovim limfomom, tipa nodularne limfocitne predominacije je od značaja radi adekvatnog postavljanja dijagnoze i odluke o terapijskom modalitetu.

Ključne reči: Hočkinova bolest; B-limfociti; dijagnoza; morfološki i mikroskopski nalazi; terapija; ishod lečenja

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Abbreviations

NLPHL – nodular lymphocyte predominant Hodgkin lymphoma
HL – Hodgkin lymphoma
CAC – The Clinical Advisory Committee
cHL – classical Hodgkin lymphoma
THRLBCL – T-cell/histiocyte-rich large B-cell lymphoma
MSCT – multi-slice computed tomography
CNB – core needle biopsy
LP cells – lymphocyte predominant cells
R CHOP – rituximab, cyclophosphamide, prednisolone, vincristine and doxorubicin protocol
PET/CT – positron emission tomography/computed tomography
DHAP protocol– dexamethasone, cytarabine and cisplatin protocol
NLPBL – nodular lymphocyte predominant B-cell lymphoma
DLBCL – diffuse large B cell lymphoma
LRCHL – lymphocyte-rich classic Hodgkin lymphoma
EBER – Epstein-Barr virus-encoded small RNAs
ABVD protocol– doxorubicin, bleomycin, vinblastine, dacarbazine
BEACOP Pesc. protocol – bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone
GHSG – The German Hodgkin study group
PFS – progression-free survival OS - overall survival
ORR – overall response rate

Introduction

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a rare type of a lymphoma that appears in only 5% of Hodgkin lymphomas (HL) [1–3]. NLPHL can present with different histopathological growth patterns [4]. The conclusion of the Clinical Advisory Committee (CAC) conference is that new terminology is warranted for NLPHL, since there are differences between classical Hodgkin lymphoma (cHL) and NLPHL, but in contrast there is a close relationship to T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) [5].

In this study we report a case of NLPHL with THRLBCL pathohistological characteristics and extranodal presentation, with a look at the pathohistological variants in NLPHL and its implication on treatment management.

Case presentation

In a 39 years-old male patient, focal changes in spleen were discovered during the routine ultrasonography of the abdomen. Further, a multi-slice computed tomography (MSCT) of the thorax and upper abdomen was performed. The MSCT showed a solid mass in the front wall of the right hemithorax with an approximate diameter of 150 x 55 x 100mm. The mass was spreading intrathoracically and disseminating into intercostal muscles, with possible infiltration of the 6th and 7th rib. Spleen enlargement (diameter 155 x110 mm) with numerous hypodense focal changes and retroperitoneal lymphadenopathy were also observed.

After the physical examination and initial laboratory analysis, that were both within reference ranges, core needle biopsy (CNB) of the mass was performed. The pathohistological analysis of the CNB showed a predominantly nodular tumor infiltrate consisting of T-cells in many nodules and intermingled lymphocyte predominant (LP) cells, consistent with pattern D (T-cell–rich nodular) NLPHL per Fan et al. [4]. LP cells were positive for PAX-5, CD20, OCT-2, Ki 67 and negative for CD30 and CD15. The patient had been referred to a hematologist and soon afterwards was hospitalized for further diagnostic and therapeutic evaluation. Bone marrow biopsy was performed and pathohistological analysis excluded bone marrow infiltration with lymphocytic cells.

Considering the aggressive presentation with extranodal infiltration and bulky mass, a chemotherapy combination of cyclophosphamide, prednisolone, vincristine and doxorubicin (CHOP) along with rituximab (R) was initiated. The patient received 6 cycles of R-CHOP immunochemotherapy protocol, which resulted in MSCT and laboratory confirmed partial remission of the disease. In addition, the whole body positron emission tomography/computed tomography (PET/CT) that was performed after the first therapy line did not show significant metabolic activity.

Six months after the initial treatment, the relapse of the disease was observed radiologically, with hepatomegaly, splenomegaly and sub-pleural infiltration in the projection of the 9th rib. Following this, salvage therapy with dexamethasone, cytarabine and cisplatin (DHAP) chemotherapy protocol was initiated and resulted in partial remission after 4 cycles of therapy.

Control whole body PET/CT scan was performed after the second line of therapy and no signs of metabolic activity were observed.

Discussion

NLPHL is an uncommon type of HL that occurs in only 5% of HL and usually presents with an indolent clinical course [1–3]. The conclusion of the CAC conference is that new terminology is needed for NLPHL, because of major biological and clinical differences with cHL and close relationship to THRLBCL. The term ‘nodular lymphocyte predominant B-cell lymphoma’ (NLPBL) is accepted by consensus [5].

Some authors have found that a surgical biopsy remains the gold standard for the initial diagnosis of NLPHL [6]. The diagnosis of NLPHL in CNB is particularly challenging given the limited tissue architecture. Small biopsy samples that include sufficient intact architecture can increase the difficulty of differential diagnosis with cHL and THRLBCL [7].

NLPHL can present with different histopathological growth patterns [4]. The presence of LP cells with preserved B-cell phenotype and a lack of CD30 is the prerequisite for the diagnosis of NLPHL [8, 9]. LP cells are typically embedded in large nodules of B lymphocytes (growth patterns A and B), but variants that are characterized by LP cells located

outside the nodules, a T-cell–rich nodular growth pattern and T-cell–rich or B-cell–rich diffuse growth patterns, respectively, have also been described (growth patterns C, D, E, and F). Variant growth patterns are associated with the recurrence and progression of disease and should be recognized and specified in pathology reports. These patterns also provide a conceptual framework for the diagnosis and further study of the biologic continuum between NLPHL and THRLBCL/diffuse large B-cell lymphoma (DLBCL), as well as cHL [7].

The main morphologic mimic of NLPHL is lymphocyte-rich classic Hodgkin lymphoma (LRCHL), which is distinguished by weak to absent B-cell antigen expression, such as CD20, consistent expression of CD30, frequent CD15 expression, and in situ hybridization staining for Epstein-Barr virus-encoded small RNAs (EBER) in approximately 40% to 50% of cases [10].

The diffuse variant (pattern E per Fan et al.) of NLPHL can be very difficult to distinguish from THRLBCL [4]. Broad B-cell immunohistochemical panel, including PAX5, CD79a, Bob.1, and Oct-2, is indicated in these cases to distinguish between NLPHL, LRCHL, and THRLBCL which have significant differences in clinical behavior and treatment [1]. A multidisciplinary approach is beneficial in order to optimize diagnosis and management of patients with this spectrum of lymphomas [7].

There is a correlation between the pathohistological type of NLPHL and its clinical presentation. In cases of NLPHL that have histopathological resemblance with THRLBCL, a more aggressive clinical presentation, including liver and spleen infiltration, can be expected, unlike the indolent clinical course that is common for other types of NLPHL [1, 11–13]. Most of the patients with NLPHL, 80% of them, have limited stage of the disease. On the contrary, advanced stage is seen in patients with NLPHL THCRBCL – like histological type [13–16].

There are different treatment approaches in patients with NLPHL depending on pathohistological type, clinical presentation and stage of the disease. Treatment may include active surveillance, radiation therapy, immunotherapy or chemotherapy [1, 11, 14]. Variant histology patterns, A, B and C or Grade 1, and D, E and F or Grade 2, along with clinical features, should be considered while deciding on the therapy course. The grade 2 histology type may deserve treatment as DLBCL [4, 5]. However, the treatment choice based on clinical features remains the main recommendation in the CAC consensus [5].

Patients in the early stage of the disease and present symptoms are usually treated with chemotherapy including the ABVD protocol (doxorubicin, bleomycin, vinblastine, dacarbazine) in the first therapy line in combination with radiotherapy. In advanced stages, the therapy choice is a more aggressive approach, including BEACOPPesc. protocol (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone), or administration of the R-CHOP protocol [1, 11, 14, 17, 18]. A report from The German Hodgkin Study Group (GHSG) in 2013. that included 413 cases, studied differences between typical and variant histopathological patterns and their prognostic impact. In 63 cases (15.3%) a variant histopathologic growth pattern (C, D, E or F pattern) was observed. Progression-free survival (PFS) at 5 years was better for patients without the mentioned histopathologic variant, with statistical significance, but differences between 5-year overall survival (OS) did not have statistical significance. The report from GHSG suggested a combined clinical and histopathologic prognostic score composed of histopathologic pattern, albumin and gender, with the significant correlation with the 5-year progression/relapse rates [19].

In a retrospective study in 2017. that included 59 patients with NLPHL, 27 patients had received R-CHOP protocol, 4-6 cycles, with overall response rate (ORR) of 100% and 88.5% estimated 5 year PFS [18].

In some patients, despite the indolent character of the NLPHL and administration of therapy, progression or relapse of the disease occurs [1, 11, 14, 16]. According to a retrospective analysis of GHSG, the prognosis for patients with NLPHL is at least as good as that for patients with cHL, but the rate of relapse is higher [1, 13, 16, 19–21]. In the previously mentioned study, one patient with NLPHL and THRLBCL that was treated with R-CHOP protocol, had progression within 1 year. (18) In NLPHL the incidence of transformation into DLBCL is higher than in HL [11, 22, 23]. Transformation is more likely in patients with advanced stage, with spleen involvement, despite aggressive treatment [22, 24]. Therefore, in all patients with relapse, repeat biopsy and pathohistological examination is necessary.

In patients with relapse according to previous studies, monotherapy with R showed promising results, along with autologous stem cell transplantation [1, 14, 17].

Conclusion

Pathohistological analysis of tissue biopsy with variant pattern evaluation along with the clinical features should be considered in therapy assessment of for patients with nodular lymphocyte predominant Hodgkin lymphoma. A multidisciplinary approach is beneficial in order to optimize diagnosis and management. Thus, nodular lymphocyte predominant Hodgkin lymphoma represents a lymphoma entity with distinct pathological characteristics that should influence treatment decisions in patients diagnosed with this rare disease.

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MULTIPLE MYELOMA
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REAL-WORLD EVIDENCE IN DIAGNOSTICS AND TREATMENT OF PATIENTS WITH MULTIPLE MYELOMA

"REAL WORLD EVIDENCE" U DIJAGNOSTICI I LEČENJU BOLESNIKA SA MULTIPLIM MIJELOMOM

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Summary

Introduction. Multiple myeloma is the second most common malignant hemopathy. The average incidence of this disease in our country is 4.2-6.7/100,000 inhabitants per year. Despite significant progress in the overall survival over the past 20 years, only 10-15% of patients achieve long-term remission and an average survival that corresponds to the average life expectancy of the general human population. The current recommendations for the treatment of multiple myeloma are globally based on the results of randomized clinical trials in which only 10-40% of patients are treated, which is why the analysis of the results of the patients treated outside of randomized clinical trials, the so-called "Real-World Evidence" data, is very important. **Diagnosis and treatment of multiple myeloma - Real-World Evidence.** Through presentations of the cases from routine clinical practice, multiple myeloma treatment modalities are presented in accordance with modern recommendations for diagnosis and treatment, as well as the multiple myeloma phase of the disease: treatment approach in newly diagnosed patients; treatment of the first relapse; and treatment of the second and subsequent relapses. **Conclusion.** The analysis of Real-World Evidence data indicates significant differences between clinical and laboratory characteristics of patients treated outside randomized clinical trials in comparison to the randomized clinical trials participants, with an significant impact to the therapeutic approach, course and outcome of the disease, consequently leading to the necessity of further *Real-World Evidence* analyzes and their inclusion in the current recommendations for multiple myeloma diagnosis and treatment

Key words: Multiple Myeloma; Diagnosis; Therapeutics; Prognosis; Recurrence; Data Analysis; Treatment Outcome

Sažetak

Uvod. Multipli mijelom je druga po učestalosti maligna hemopatija. Prosečna incidencija ove bolesti u našoj zemlji je 4,2−6,7/100.000 stanovnika godišnje. I pored značajnog napretka u ukupnom preživljavanju u poslednjih 20 godina, samo 10−15% bolesnika postiže dugotrajnu remisiju i prosečno preživljavanje koje odgovara prosečnoj dužini života opšte ljudske populacije. Aktuelne preporuke za lečenje multipliog mijeloma zasnovane su na rezultatima kliničkih istraživanja u okviru kojih se leči svega 10−40% bolesnika, zbog čega su analize rezultata lečenja bolesnika izvan randomizovanih kliničkih istraživanja, tzv. *Real-World Evidence* podaci, veoma važni. **Dijagnostika i lečenja multiplog mijeloma –** *Real-World Evidence***.** Kroz prikaze slučajeva iz kliničke prakse, predstavljeni su modaliteti lečenja multiplog mijeloma u skladu sa savremenim preporukama za dijagnostiku i lečenje i fazom bolesti: terapijski pristup kod novodijagnostikovanih bolesnika; terapija u prvom relapsu; terapija u drugom i sledećim relapsima. **Zaključak.** Analiza *Real-World Evidence* podataka ukazuje na značajne razlike u kliničko-laboratorijskim karakteristikama bolesnika lečenih izvan randomizovanih kliničkih istraživanja, koje su od uticaja na terapijski pristup, tok i ishod bolesti, sa posledičnom neophodnošću sprovođenja daljih analiza *Real-World Evidence* i njihovo uključenje u aktuelne preporuke za dijagnostiku i lečenje mutiplog mijeloma.

Ključne reči: multipli mijelom; dijagnoza; terapija; prognoza; relaps; analiza podataka; ishod lečenja

Introduction

Multiple myeloma (MM) is the second most common malignant hemopathy [1]. The average incidence of this disease in our country is 4.2-6.7/100.000 inhabitants per year [2]. Despite significant progress in overall survival (OS) over the past 20 years, only 10-15% of patients achieve long-term remission and an average survival that corresponds to the average life expectancy of the gen-

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Abbreviations

VCD protocol	– Velcade®-Cyclophosphamide-Dexamethason protocol
VAD protocol	– Vincristin-Adriablastine-Dexamethason protocol
TAD protocol	– Thalidomide- Adriablastine-Dexamethason protocol
VTD-Velcade® protocol	– Thalidomide-Dexamethason protocol
Dara-VTD protocol	– Daratumumab-Velcade®-Thalidomide-Dexamethasone protocol
Vel-Dex protocol	– Velcade®-Dexamethasone protocol

eral human population [3]. Current recommendations for the treatment of MM are based on the results of randomized clinical trials (CRT), in which only 10-40% of patients are treated, which is why the analysis of the results of the patients treated outside of RCT, the so-called "Real-World Evidence" (RWE) data, is very important [4-6].

Through three typical case reports from the current clinical practice, current treatment approach in MM patients is presented in accordance with modern recommendations for diagnosis and treatment: treatment of newly diagnosed (NDMM) patients; therapy in the first relapse; and treatment approach in the second and subsequent relapses [7–9].

Diagnosis and treatment of multiple myeloma - RWE

Case report: Therapeutic approach to newly diagnosed patients

A 47-year-old female patient was referred to a hematologist at the Clinical-Hospital Center (CHC) "Zvezdara" in October 2020, due to anemia, elevated sedimentation, hypercalcemia and back pain that lasted for several months. After the initial examination, a diagnosis of IgG kappa MM in IIIA clinical stage of disease (CS, Durie&Salmon) was established, with high-risk ISS score 3, and Revised-ISS (R-ISS) score 3 as well [10–14]. Performed fluorescence in-situ hybridization (FISH) revealed the presence of deletion 13q14 (del13x14) [15]. Treatment was started with high-doses dexamethasone, followed with chemotherapy (HT) according to the VCD protocol with bortezomib [7, 8, 15]. Due to the appearance of respiratory infection, accompanied by fever and inflammatory syndrome, the presence of a COVID 19 infection was registered by PCR testing, which is why the patient was transferred to the COVID-19 Center of CHC "Bezanijska kosa" for further treatment. On admission, the patient had dispnea and auscultatory findings of late-inspiral crackles in the middle lung fields and room air saturation of 80%. Laboratory findings showed normocytic anemia (Hgb 95g/l, MCV 89fL), leukopenia with lymphopenia (WBC 2.87; Ly 0.68x10^9/l), while biochemical findings showed elevated levels of IL-6 (108.1 pg/ml), ferritin (806 ug/l), LDH (83 1U/l), CRP (135.6 mg/l), procalcitonin (0.18 ng/ml), D dimer (851 ng/ml), and creatinine (104 mmol/l), with normal values of calcium (1.69 mmol/l), total protein (60 g/l) and albumin (36 g/l). CT scan of the chest revealed "milk glass" opacifications and consolidation of the lung parenchyma bilaterally, with a CT "severity" score of 25 (maximum score 25); CO-RADS almost 6, stage: PIK [16, 17]. In addition to empiric antibiotic therapy and anticoagulant therapy, virulostatic therapy with Remdesivir is included as well. The application of pulse doses of dexamethasone was also continued [18, 19]. Due to the progressive worsening of the COVID 19 pneumonia from the sixth day of detected infection, patient was transferred to the intensive care unit where non-invasive ventilation was applied, along with administration of tocilizumab, a humanized monoclonal antibody that blocks membrane receptors for IL-6, after which the general condition gradually improved [20]. During recovery the period, inflammation parameters decreased and bilateral pneumonia regressed, while maintaining dependence on oxygen support and the necessity of using an oxygen concentrator in home conditions.

Following the recovery from COVID 19 pneumonia in January 2021, the MM was started according to the VCD protocol in CHC "Zvezdara" [7, 8, 18]. The last VI cycle of therapy was applied in May 2021 with the effect of partial remission (IMWG criteria, PR). During July 2021, the patient was diagnosed with signs of disease progression (PD) [21]. Hematological evaluation revealed massive infiltration in the sample of bone marrow biopsy with monoclonal plasma cells (90%), and the presence of high-risk chromosomal abnormalities by an control FISH analysis: del17p in 50% of nuclei; amplification 1q21 in 20% of nuclei, with 4p16 polyploidy in 50%; and 16q23 in 50% of nuclei [7, 8, 14, 15]. The treatment was continued according to the TAD protocol, accompanied by the occurrence of thrombosis along superficial femoral vein and the popliteal vein of the right leg. Thalidomide was excluded from the therapy, and the treatment was continued using the standard VAD protocol. In January 2022, further treatment was continued in the program of high-dose HT followed by autologous stem cell transplantation (ASCT) [7, 8]. During hospitalization for the purpose of mobilization and collection of hematopoietic stem cells (HSC) using mobilizing HT according to the CAD protocol in combination with granulocyte-colony stimulating growth factor (G-CSF), fever caused by PCR-proven COVID 19 infection occurred, and the patient was transferred to the CHC "Batajnica" for the treatment of COVID 19 re-infection. After recovery, in April 2022, "steady state" mobilization was carried out with a combination of G-CSF and plerixafor with collection and cryoconservation of 9.5 x 10^6/kgTT CD34+ [7, 8, 22]. By the end of May 2022, treatment with high-dose Melphalan (200 mg/m^2) supported by ASCT. The period of post-transplant recovery was complicated due to the occurrence of pulmonary aspergillosis and slower hematopoietic recovery, followed by the achievement of criteria for a very good partial remission (IMWG criteria, VGPR) on the +100. day after ASCT [21, 22].

Discussion 1: In accordance with international recommendations adjusted to the variations of different health programs and guidelines proposed by Serbian Myeloma Group (SMG), the decision on the initial therapeutic approach to NDMM patients is based on the eligibility for ASCT, individual clinical characteristics and prognostic profile of the patient. The ultimate goal of treatment of NDMM patients younger than 65 years who are transplant-eligible, is to achieve long-term remission and potential cure. The standard first line therapy for these patients consists of: 1) triple HT bortezomib based combinations with immunomodulatory drugs (VRD with lenalidomide, VTD with thalidomide), or with standard cytostatics (CVD with cyclophosphamide; PAD with doxorubucun); or 2) triple thalidomide based combinations (CTD or TAD) [7, 8, 23]. RWE data indicate that initially 44-60% of NDMM patients are considered as transplant-eligible, while this type of treatment is actually carried out in 31% of NDMM patients [4–6]. Our patient was treated with a triple VCD combination, after which a short-term therapeutic response was achieved, which is probably a consequence of the development of resistance caused by clonal evolution and the accumulation of high-risk cytogenetic aberrations that were detected during disease progression, indicating the importance of repeated FISH analysis for the treatment approach in relapsing/refractory (RRMM) patients with standard risk features at diagnosis [9, 24, 25]. According to the SMG recommendations, the optimal treatment of ultra-high-risk NDMM patients ("double/triple-hit" MM, R-ISS 3 with high-risk chromosomal abnormalities and high LDH, plasmacytic leukemia) consists of quadruple bortezomib based combinations with immunomodulatory drugs and monoclonal antiCD38 antibody (Dara-VRD, Dara-VTD). The so-called "tandem" ASCT should be performed out during first 3-12 months after the first ASCT, in high-risk patients (R-ISS 2 and 3), or in the case of PR as a maximum response after the first ASCT [7, 8]. The goal of treatment of NDMM patients older than 65 year, and transplant-ineligible NDMM patients, is to prolong OS while maintaining a good general condition and achievement of independence from the treatment/care in hospital conditions, with the application of various triple and quadruple combinations of bortezomib, and/or immunomodulatory drugs with the antiCD38 monoclonal antibody daratumumab (R-ISS 2 and 3) in high-risk patients [7, 8, 23].

Patients with hematological malignancies have a particularly high risk of developing severe and life-threatening infections, due to immunodeficiency caused by the disease itself, as well as due to immunosuppressive therapy. According to the results of studies published so far, the total mortality rate in patients with hematological malignancies and COVID 19 infection is from 28%, up to 42% among patients who require hospitalization [26, 27]. The highest rates of morbidity and mortality were recorded in senior patients, during swing of malignant disease, with impaired renal function and associated diseases such as hypertension and diabetes, or expected OS shorter than 12 months at the time of COVID 19 infection [28, 29]. Our patient had a severe form of COVID 19 infection in which tocilizumab was administered in the life-threatening phase. The IL 6 signaling pathway is crucial in the pathogenesis of multiple myeloma, as well as during "cytokine storm" caused by COVID 19 infection. Considering the severe clinical picture, deterioration of respiratory function, progression of lung changes and high IL 6 value, tocilizumab was administered to our patient with a successful outcome. In accordance with limited experiences, the use of tocilizumab in patients with a malignant disease and COVID 19 infection is still controversial, due to its immunosuppressive effect and the possibility of developing secondary bacterial infections [20].

Case report: Therapy in the first relapse of multiple myeloma

A female patient, aged 38, was first diagnosed with multiple myeloma in April 2012. Initial diagnosis and treatment were carried out at the Military Medical Academy. Among the complaints, she had weakness and pain in the thoracic spine, and they persisted 3 months before the diagnosis was established. Due to the findings of the MRI examination of the thoracic spine, a laminectomy of the second and third thoracic vertebra with extirpation of the tumor and decompression of the spinal canal was performed first. Pathohistological and immunohistochemical findings of the removed tumor indicated plasmocytoma, followed by a complete hematological examination. Laboratory analyzes showed normocytic anemia (Hgb 92 g/l, MCV 94fL) and 24 h proteinuria of nephrotic grade 8.4 g/24 h, without accelerated sedimentation, azotemia, hyperproteinemia, hypercalcemia, with a normal LDH value. Serum protein electrophoresis indicated the presence of paraprotein, without a typical peak, and immunoelectrophoresis identified monoclonal lambda light chains while urine protein electrophoresis with immunoelectrophoresis identified the presence of monoclonal lambda light chains. A bone marrow biopsy was performed and the pathohistological findings with immunohistochemistry confirmed the diagnosis of multiple myeloma based on monoclonal plasma cells (CD38+, lambda+, kappa-) infiltration of 60%. The patient was diagnosed with Myeloma multiplex BJ lambda CS IIIA, ISS1 and she was initially treated with four cycles of chemotherapy according to the VAD protocol with palliative radiotherapy of the thoracic spine, with less than partial remission, with maintenance of residual bone marrow infiltration (30%) and persistence of proteinuria [8,10–14]. She was then included in a program of high-dose chemotherapy, followed by autologous hematopoietic stem cell transplantation [7,8]. The effect of the treatment was a long-term complete remission (CR), with the application of maintenance therapy with thalidomide during 12 months after ASCT [8, 21]. In April 2022, the patient was examined for the first time by a hematologist at the Clinic of Hematology University

Clinical Center of Serbia, with laboratory finding indicative for relapse, observed at regular check-up. She had no subjective complaints. Laboratory analyzes showed mild leukopenia (WBC 3.5 x 10^9/l), no anemia (Hgb 132 g/l, MCV 97fL), and normal platelet count (Plt 214 x 10^9/l). Biochemical analyzes revealed the absence of accelerated sedimentation (6/), azotemia, hyperproteinemia (total proteins 68 g/l), hypoalbuminemia (albumins 47 g/l), hypercalcemia (Ca 2.39 mmol/l), elevation of LDH (LDH 187 U/l) or Beta2 microglobulin concentration (2.35 mg/l). Along with low concentrations of lambda light chains detected by serum imunoelectrophoresis, determination of the serum free light chains showed high concentration of free lambda light chains (315 mg/L) accompanied with normal concentration of free kappa light chains (10.7 mg/l) and a significantly reduced kappa/lambda ratio of 0.03. The 24h proteinuria was 0.81 g/24h and presence of monoclonal lambda light chains were identified by urine protein immunoelectrophoresis. The patient underwent a bone marrow biopsy and the pathohistological findings with immunohistochemistry indicated monoclonal plasma cell infiltration of 60%. FISH analysis revealed del13q14 in 50% nuclei, amplification of 1q21 in 30% nuclei, along with polyploidy of 17p13, 14q32, and 4p16. CT scan of the axial skeleton showed multiple osteolytic changes in the bones of the axial skeleton. Based on the performed hematological diagnostics, the first relapse of Myeloma multiplex BJ lambda CS IIIA, ISS1, R-ISS1 was confirmed [8, 10–14]. In accordanco with the evolving character of the disease, due to the clonal vulnerability by appearance of amp1q21 in relapse, consequently accompanied with accumulated genetic events, treatment was initiated with bortezomib based triplet VTd in combination with monoclonal antiCD38 antibody, daratumumab (Dara-VTd) from the second cycle of treatment [30, 31]. The assessment after two cycles of therapy (I cycle of VTd and I cycle of Dara-VTd) indicated achievement of PR (reduction of 24 h proteinuria 0.1 g/24 and concentration of free lambda light chains 109 mg/l) [21]. So far, the patient completed 3 cycles of treatment according to the Dara-VTd protocol, maintaining the treatment response of PR. In accordance wite transplant eligibility and previous long-term remission, secondary salvage ASCT is planned after 4-6 cycles of induction chemotherapy [7, 31].

Discussion 2: Incorporation of new treatment modalities with different mechanisms and targets of action as proteasome inhibitors, immunomodulatory drugs, and immunotherapeutic modalities as monoclonal antibodies, chimeric-antigen T (CAR-T) cells, antibody-drug conjugates, or bispecific T-cell engagers (BiTEs), completely changed the MM landscape providing the possibility of transformation of MM from an incurable disease to a well-controlled chronic state [32]. In this view, treatment approach in MM relapse is based on several key-points, as: duration of previous remission; individual prognostic profile; and transplant eligibility. In accordance to un/availability of lenalidomide maintenance, in case of durable previous remission (≥ 2 years), the previous therapy may be repeated. Ie case of remission shorter than 2 years, new treatment modalities should be applied, adjusted to the initial treatment [7–9, 24, 25, 33]. All relapsed patients should be re-stratified according to the R-ISS score, and new prognostic score Revised 2 ISS score (R2-ISS) incorporating ISS score, LDH elevation, translocation t(4;14), del17p, and amp1q21 [34]. According to the R2-ISS, our patient is stratified as low-intermediate with R2-ISS 2. High-risk features of the disease as hypo-secretory immature characteristics with accumulation of genetic abnormalities including amp1q21 and extensive bone disease, indicated the necessity of bortezomib based triplet with immunomodulatory drug in combination with monoclonal antiCD38 antibody, daratumumab [30, 34, 35]. Previous long-term durable remission may be subject of discussion in terms of MM relapse caused by clonal evolution, or occurrence of new genetic events triggering new MM clone, resulting with the treatment approach as in NDMM patients [30] in Considerine RWE data, treatment decision in RRMM patients should incorporate factors with impact to the patients reported outcomes, such as: patient preferences, physical activity, work productivity, comorbidities, quality of life, disease symptoms/control, treatment-related toxicity, treatment convenience, and so-called "financial toxicity" [36]. Considering treatment choice, RWE data indicates the gap between RCT results and RWE analyses regarding treatment efficacy among triplets of new treatment modalities (bortezomib, carfilzomib, ixazomib, daratumumab) witn a lenalidomide-dexamethasone as backbone [37].

Case report: Treatment approach in the second and subsequent relapses

A female patient, 55 years old, in September 2019. was referred to an initial hematological examination due to accelerated sedimentation rate and anemia. Given symptoms included moderate general weakness, while health history included an acute myocardial infarction four years prior. The insight into basic laboratory parameters, verified an elevated level of serum proteins and hypercalcemia, so an additional diagnostic panel was expanded according to the algorithm for multiple myeloma. Normal kidney function was observed, but an M component in serum protein electrophoresis was detected as well as an elevated serum level of IgA (30 g/l) accompanied with lower levels of IgM and IgG. Using protein immunofixation of the serum an IgA lambda M component was isolated and from the 24 hour urine lambda light chains were observed. The initial FISH analysis showed no high risk cytogenetic abnormalities. The pathohistological and immunohistochemical analysis of the bone marrow bioptate showed an infiltration with 80-90% of monoclonal, CD38+, kappa-, lambda+, plasma cells. Due to the absence of bone disease signs by standard X-Ray examination, a PET-CT was performed indicating metabolic activity of discrete osteolytic lesions in sternum, bilateral edges of 7. ribs, thoracic and lumbar spine as well as pelvic. Based on such findings, the diagnosis of Multiple myeloma IgAλ, CSIIIA ISS1

R-ISS1 was established [7, 8, 10–14, 38]. Induction therapy was started according to the VCD protocol and after 4 cycles, VGPR was achieved. In accordance with the age of the patient and transplant eligibility, HSC mobilization, collection and cryopreservation were performed. The induction treatment was completed by 6 cycles of chemotherapy. Due to the high-spread of COVID 19 pandemic, high-dose chemotherapy and ASCT were postponed, and patient's follow-up was performed from May to September of 2020. In September 2020, treatment with high-dose melphalan (200mg/m^2) and ASCT was continued, with confirmed treatment response of VGPR on +10. day after ASCT [7, 8, 21, 22]. Still, already in March 2021, four months after the ASCT, the patient reported pain in the lumbar region during an ambulatory control. A supplemental hematological diagnostic was performed, confirming a relapse of the disease. The treatment of the first relapse of the disease was started according to the lenalidomide-dexamethason protocol. During the 3. cycle of treatment, the clinical course was complicated by the development of non-COVID pneumonia. Following the patient's recovery, the control laboratory diagnostics confirmed further PD [21]. Considering the expressed lenalidomide refractoriness and bortezomib sensitivity, further treatment was continued with bortezomib-based combination with daratumumab and dexamethason according to DVd protocol. In accordance to the health-carellawful regulative, after application of 4 cycles according Vel-Dex doublet and 4 cycles of DVd protocol, resulting with PR achievement, treatment was continued by administering daratumumab, but in combination with cyclophosphamide and dexamethason. However, further PD was noticed in the following 3 months, while control FISH analysis detected occurrence of amp1q21. Following patients re-staging indicated R2-ISS score of medium-high risk [21, 24, 25, 34].

Discussion 3: As stated above, multiple factors have significant impact to the treatment choice in MM relapse, as well as the course of treatment and outcome, including current COVID 19 pandemic related to the maintenance of the patient's quality of life. In this view, new treatment modalities provide powerful control of the disease accompanied by restitution of immune system and maintenance of qualitative improvements in daily activities [7–9, 25, 39, 40]. The course of treatment and its efficacy in our patient was compromised due to the high-spread of COVID 19 pandemic. Furthermore, re-staging of disease in the relapse of patients initially staged as of standard risk, represents *sine qua non* for the adequate treatment approach [41].

Conclusion

The analysis of Real-World Evidence data indicates significant differences between clinical and laboratory characteristics of patients treated outside randomized controlled trials in comparison to the randomized controlled trial participants, with an significant impact to the therapeutic approach, course and outcome of the disease, consequently leading to the necessity of further Real-World Evidence analyzes and their inclusion in the current recommendations for multiple myeloma diagnosis and treatment.

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MYELOPROLIFERATIVE NEOPLASMS
MIJELOPROLIFERATIVNE NEOPLAZME

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MYELOPROLIFERATIVE NEOPLASMS AND PREGNANCY

MIJELOPROLIFERATIVNE NEOPLAZME I TRUDNOĆA

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Summary

Introduction. The Myeloproliferative Neoplasms are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by increased proliferation of the myeloid lineages in the bone marrow. A particular clinical challenge is presented by certain situations in patients with myeloproliferative Neoplasms, which we do not encounter daily. For this reason, in this paper, we will emphasize the approach to overcoming obstacles in patients with Myeloproliferative Neoplasms in specific settings, like pregnancy. Pregnancy with Philadelphia chromosome-negative Myeloproliferative Neoplasms has been reported to be associated with maternal thrombosis, hemorrhage, and placental dysfunction leading to fetal growth restriction or loss. Thrombocytosis, leucocytosis, high level of hematocrit, activation of Platelets, leucocytes, and circulating pro-thrombotic are connected with the pathogenesis of thrombosis in MPNs With survival expectations similar to age-matched controls and excellent response and worldwide access to tyrosine kinase inhibitors, family planning is increasingly important for many patients with chronic myeloid leukemia. All patients were managed by a multidisciplinary team of physicians with obligatory hematological and gynecologists-obstetrician consultations.

Key words: Myeloproliferative Disorders; Neoplasms; Philadelphia Chromosome; Pregnancy; Risk Factors; Thrombosis; Therapeutics; Patient Care Team; Pregnancy Outcome

Sažetak

Uvod. Mijeloproliferativne neoplazme su heterogena grupa poremećaja klonskih hematopoetskih matičnih ćelija koje karakterišu povećana proliferacija mijeloidnih linija u koštanoj srži. Poseban klinički izazov predstavljaju određene situacije kod bolesnika sa mijeloproliferativnom neoplazmom, a sa kojima se ne susrećemo svakodnevno. Zbog toga ćemo u ovom radu staviti naglasak na pristup u savladavanju prepreka kod bolesnika sa mijeloproliferativnom neoplazmom u specifičnim okolnostima, poput trudnoće. Poznato je da je trudnoća kod *Filadelfija* hromozom-negativnih mijeloproliferativnih neoplazmi povezana sa trombozama kod trudnica, krvarenjem i disfunkcijom placente, što dovodi do ograničenja fetalnog rasta ili njegovog gubitka. Više činilaca verovatno doprinosi patogenezi tromboze u mijeloproliferativnim neoplazmama; trombocitoza, leukocitoza, visoka vrednost hematokrita, aktivacija trombocita i leukocita, te cirkulišući protrombotski i endotelni faktori. Primena infibitora tirozin kinaze kod bolesnika sa hroničnom mijeloidnom leukemijom, doprinela je da je očekivano preživljavanje ovih bolesnika promenjeno, a planiranje porodice postaje sve važnije za značajan broj ovih bolesnika. Zbog toga je za uspešan ishod trudnoće u mijeloproliferativnim neoplazmama neophodan multidisciplinarni tim lekara uz obavezne hematološke i ginekološko-akušerske konsultacije.

Ključne reči: mijelodisplastični poremećaj; tumori; Filadelfija hromozom; trudnoća; faktori rizika; tromboza; terapija; multidisciplinarni tim; ishod trudnoće

Introduction

The Myeloproliferative Neoplasms (MPNs) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by increased proliferation of the myeloid lineages in the bone marrow (BM) [1]. The MPNs are traditionally classified into four sub-groups, which include Chronic Myeloid Leukemia (CML), classical Philadelphia-negative MPNs (Polycythemia Vera, PV; Essential Thrombocythemia, ET; Primary Myelofibrosis, PMF), non-classical Philadelphia-negative MPNs (Chronic Neutrophilic Leukemia, CNL; Chronic Eosinophilic Leukemia, CEL) and MPN, not otherwise specified [1, 2]. A particular clinical challenge is presented by certain situations in patients with MPN, which we do not encounter daily. For this reason, in this paper, we will emphasize the approach to overcoming obstacles in patients with MPN in specific settings, like pregnancy.

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Philadelphia chromosome-negative MPN and pregnancy

MPN diagnoses are typically made in the sixth or seventh decade of life. Approximately 20% of patients with essential thrombocythemia and 15% with polycythemia vera are younger than 40 [3]. There is limited information on fetal and maternal outcomes and optimal management of pregnancy in patients with MPNs.

The incidence of pregnancies in patients with MPN is unknown [4]. However, based on a prospective British study, the calculated incidence of MPN pregnancies is 3,2/100 000 maternities per year [5].

However, based on a prospective British study, the calculated incidence of MPN pregnancies is 3.2/100 000 maternities per year [6].

Thrombocytosis, leucocytosis, high level of hematocrit, activation of Platelets,leucocytes, and circulating pro-thrombotic are connected with the pathogenesis of thrombosis in MPNs.

A large meta-analysis reported the outcome of 461 pregnancies in women diagnosed with ET [7]. The live birth rate was 50–70%, the first-trimester loss occurred in 25–40%, and late pregnancy losses were up to 10%. In addition, rates of intrauterine growth restriction (IUGR) were 4.5%, placental abruption (3.6%), postpartum thrombotic episodes 5.2% of pregnancies, and pre-/postpartum hemorrhage 5.2%.

The literature dates on pregnancies in PV patients are sparse. Pregnancy outcomes are described in a case series of 18 pregnancies combined with 20 historical PV patients [8].

The first-trimester loss was the most frequent complication (21%), followed by late pregnancy loss (18%), IUGR (15%), and premature delivery (13%) in PV patients [9].

Pregnancy in PMF patients is rare. The outcome reports suggest a 50% risk of fetal loss; however, no maternal complications of thrombosis or disease progression were noted, but the numbers are probably small to draw any conclusions [10].

The United Kingdom Obstetric Surveillance Survey (UKOSS) prospective data on MPN in pregnancy have shown the incidence of miscarriage was 1.7/100 pregnancies, and the perinatal mortality rate was 17/1000 live and stillbirths. Incidences of maternal complications were 8.8% (n=5/57) preeclampsia, 8.8% (n=5/57) postpartum hemorrhage, and 3.5% (n=2/57) postpartum hematoma. The results from this study have shown a higher rate of stillbirth, pre-eclampsia, cesarean section, and low birth weight in women with MPNs compared to the general population [5]. However, overall, women with MPN appear to have successful pregnancies [11].

Management of Ph-MPN and pregnancy

Women of reproductive age diagnosed with MPN should receive information about the risk and approaches to future pregnancies. Disease status, comorbidity, and prior obstetric history should involve in the future decision.

A multidisciplinary approach by an obstetrician-gynecologist experienced in high-risk pregnancies of patients with MPN and a hematologist is very important to enable optimal disease control from the preconception to the postpartum period.

Table 1 shows the factors that define high-risk pregnancy in MPN based on clinical experience.

Practical recommendation for management of MPNs in pregnancies

Maintaining platelets count less than 400 x 10^9/l, and hematocrit less than 45 g/l are two treatment aims in high-risk nonpregnant and pregnant patients with MPNs [9].

During pregnancy, close monitoring of the blood count is mandatory, every four weeks until the 24th week and then every two weeks. Blood pressure and urine analysis should be performed at every visit [9].

Preconception planning should include interruption of hydroxyurea, busulfan, and anagrelide within three to six months. Interferon alpha therapy is the treatment of choice for patients who should receive cytoreductive therapy due to disease-related reasons or in high-risk pregnancies. The recommended initial dose of Interferon alpha is 45 mcg subcutaneously weekly throughout pregnancy, with a duration of post-partum therapy based on clinical and laboratory parameters [4]. Interferon/alpha is recommended in this situations:

Table 1. High risk pregnancy in MNP patients
Tabela 1. *Visokorizične trudnoće kod bolesnica sa MSN*

Platelet count rising to >1500 × 10^9/L prior to pregnancy or during pregnancy*
Broj trombocita veći od 1500 x 10^9/l pre ili tokom trudnoće
1. Previous venous or arterial thrombosis
Prethodne venske ili arterijske tromboze.
2. The previous episode of one or more hemorrhages associated with myeloproliferative neoplasms.*
Prethodna jedna ili više epizoda krvarenja povezana za mijeloproliferativnim neoplazmama
3. Previous pregnancy complications
Prethodne komplikacije u trudnoći
4. Diabetes mellitus or hypertension required therapy
Šećerna bolest ili povišen krvni pritisak koji zahteva terapiju.
*Indication for interferon only, rather than interferon plus low molecular weight heparin [6]./*Indikacije za interferon alfa, bolje nego kombinacija interferona alfa plus heparin male molekulske težine [6].*

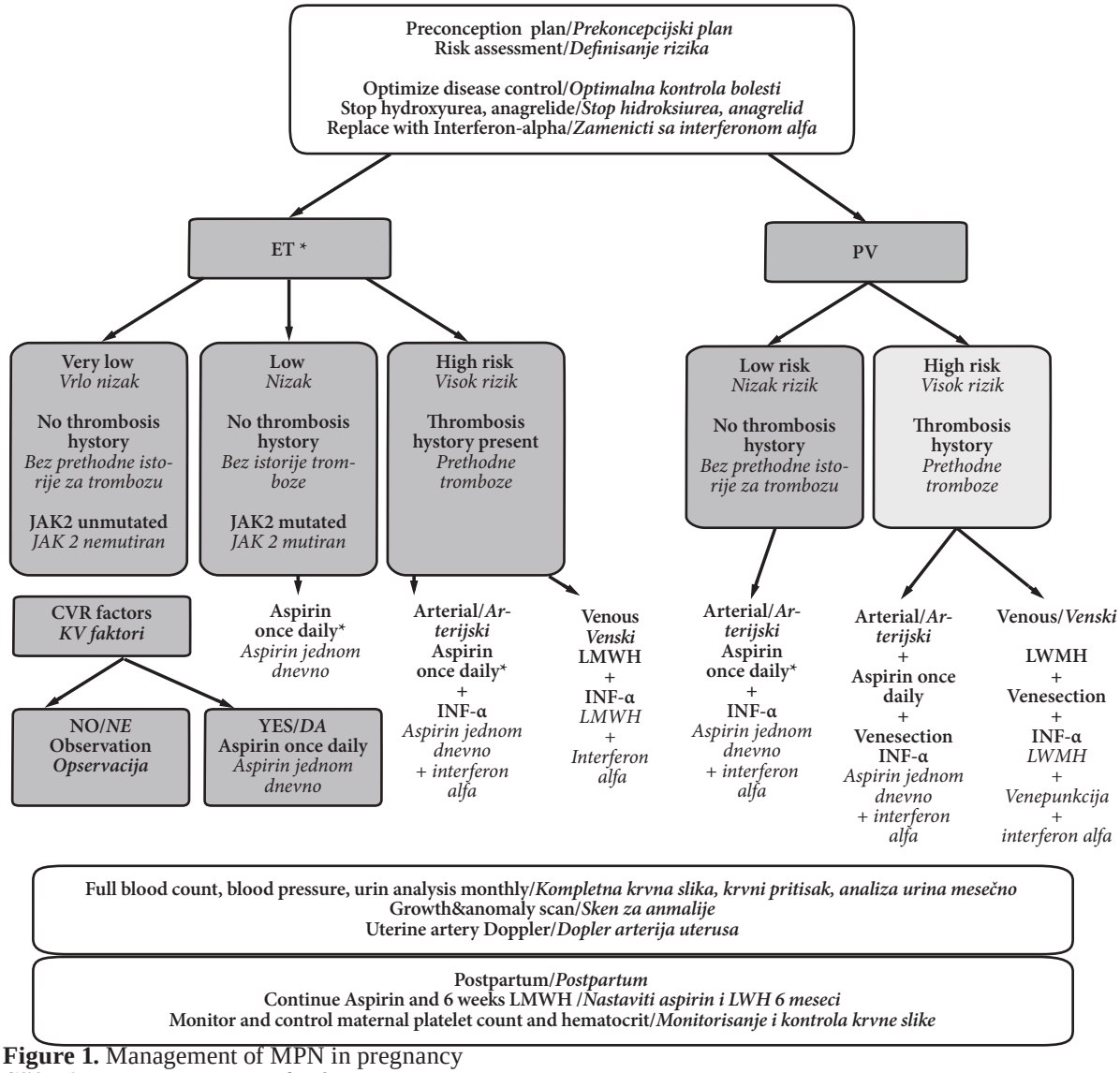

Figure 1. Management of MPN in pregnancy
Slika 1. *Pristup MPN u trudnoći*

1. Previous maternal major thromboembolic or major hemorrhagic complications requiring cytoreductive treatment before conceptions;

2.Thromboembolic events during pregnancy;

3.Uncontrolled platelets (if rising more than 1500 x 10^9/l or if hematocrit level is > 45-50%) [3].

Uterine artery Dopplers may identify women at high risk and are a predictive test for the development of pregnancy complications such as preeclampsia, intrauterine growth restriction, abruption, and fetal death. They are usually performed between 18 and 24 weeks. A systematic review and meta-analysis have shown that an increased pulsatility index is the best predictor of preeclampsia of overall and severe intrauterine growth restriction among low-risk patients [9]. In the presence of a mean pulsatility index of more than 1.4, the local practice would perform growth scans more frequently and offer escalation of treatment to include heparin and α-interferon [9].

Management of thrombotic risk

In preconception approaching, the assessment of thrombotic risk should be done, but also ongoing individual risk assessment is mandatory **(Figure 1).**

According to perceived risk, the therapeutic options include aspirin, heparin, venesection, cytoreductive agents, and thromboembolic deterrent stockings.

All patients should be treated with aspirin without clear contraindications, i.e., asthma, history of peptic ulceration, or current hemorrhage. If the platelet count is over 1000 × 10^9/L, acquired von

Willebrand disease should be excluded before commencing aspirin.

The optimal dose and schedule of aspirin therapy during pregnancy require further investigation and debate [4, 12] **(Figure 1).** INF-α therapy might also be considered in low-risk patients under certain circumstances, including the history of recurrent fetal loss, prominent splenomegaly, or suboptimal hematocrit control with phlebotomy.

Systemic anticoagulation in the form of LWMH is advised in patients with any of the high-risk MPN pregnancy factors except hemorrhage and extreme thrombocytosis.

Once adequate hemostasis has been achieved postpartum, all women should be offered six weeks of LMWH thromboprophylaxis without a prior history of a significant hemorrhage. Caution should be applied to cases where women have a history of a significant hemorrhage with a platelet count <1000 $\times 10^9$/L and no other obvious cause except for platelet dysfunction secondary to an MPN.

In PV patients, hematocrit during pregnancy should be less than < 45%. If necessary, venesection or cytoreductive therapy with interferon-alpha must be started to achieve appropriate blood counts. During pregnancy, iron supplementation is not recommended because this may cause an unpredictable rise in hematocrit and may thus increase the risk of thrombosis.

CML and pregnancy

CML management during pregnancy remains controversial. With a median age at diagnosis of over 60 years in the West, a minority of new diagnoses (approximately 20%) occur in women and men in their reproductive years [13]. Until recently, management of young patients with CML was focused on preparing for timely, curative-potential, yet high-risk allogeneic stem cell transplants. Issues related to fertility and family planning were secondary considerations.

TKIs are an effective first- or second-line therapy and have demonstrated increased overall survival [14]. In addition to BCR-ABL, TKIs inhibit other oncogenes, including PDGRF-α (platelet-derived growth factor alpha), which are essential for embryonic implantation, gonadal development, fetal maturation and are therefore associated with fetal abnormalities [15]. In this context, CML patients of reproductive age are faced with major issues of family planning with due regard to the risk of TKI treatment interruption during pregnancy. Additionally, TKI impact is another potential risk to the fetus. However, continuing this molecular targeted therapy during pregnancy, particularly throughout the first trimester, seems unsafe and harmful for the fetus, as it belongs to US Food and Drug Administration (FDA) Pregnancy Category D given this potential embryo or fetotoxicity [14].

There is a different way to manage CML if a patient already has the disease and becomes pregnant compared to patients who develop CML after becoming pregnant.

CML diagnosed during pregnancy

The diagnosis of CML during pregnancy does not necessarily lead to the initiation of treatment. The therapeutic choice of newly diagnosed CML in pregnancy requires considering termination or continuation of pregnancy, gestational stage, and disease stage. In patients with markedly increased WBC (more $100x10^9$/L) resulting in a leukostasis clinical presentation, leukocyte apheresis should be performed. IFN has a slower and less certain ability than TKI to induce control of abnormal blood counts and therefore may not be a sufficient treatment for high leukemic burden at CML onset [16].

For patients presenting in the second and third trimesters, treatment may not be necessary if the counts remain low. There is rarely an indication for hydroxycarbamide, and if used, it should only be given for a short time to reduce leukocyte or platelet counts. IFN can be used safely. In theory, imatinib can be introduced after 15 weeks (point of placental maturation and critical organ formation) because of limited placental transfer but it is not recommended by the manufacturer. Although nilotinib can be considered second-generation TKIs should be avoided until after delivery. Data regarding the teratogenicity of second-generation TKIs also point towards significant fetal toxicity, a higher rate of abortions, and abnormal pregnancies. Nilotinib may be safer than other TKIs, due to a lower placental transfer [17,18].

Pregnancy during CML treatment

Recent recommendations on CML and pregnancy management have been described after direct experience and analysis of published data [16]. The data reported confirming that in males, TKI treatment does not seem to impact fertility and conception. In contrast, female patients must stop TKI therapy as soon as possible during the first trimester of unplanned pregnancy. TKI therapy should be stopped promptly when pregnancy is discovered, during the first trimester, based on fetal risk. After an individual discussion, if pregnancy is continued, hematologic or cytogenetic relapse may be expected, and primary consideration should be given to IFN to control the disease. After placental formation and crucial fetal organ development are complete (15–16 weeks), consideration can be given to the introduction or re-introduction of select TKI therapy (imatinib or nilotinib) where indicated and after clear discussion regarding risk/benefit. Dasatinib should not be used at any time during pregnancy.

For the CML women who will either become or wish to become pregnant with an MR ≤ 2 and short exposure and suboptimal response to therapy, the recommendation is to approach similar to CML patients diagnosed during pregnancy.

The decision on the therapeutic approach to CML and planned pregnancy in patients on TKI therapy depends on the depth of the molecular response, the time from the diagnosis and duration of treatment.

Several trials have shown that the cessation of TKI in patients who attained a deep, long-lasting molecular remission might be safe and feasible with 40% of patients remaining disease-free for 24 months [19, 20].

For patients in MMR but not DMR it is not unreasonable to stop treatment to attempt conception.

Patients who have been in DMR (MR4 or better) for >12–24 months can be managed as a patient eligible for a trial of discontinuation and TFR. The recently suggested criteria for safe TKI discontinuation and observation in TFR include TKI duration for >3–4 years for the second generation TKIs or >5 years for imatinib; the recommended DMR duration is at least 1–2 years [21, 22]. These patients have a ~50% chance of being able to remain off drugs indefinitely; another 50% could lose response, the majority within 3–6 months [23]

For those patients who experience molecular relapse (MMR loss) when trying to conceive but are not yet pregnant, treatment should be re-started [16].

A patient planning a pregnancy or who conceives during CML therapy and who has achieved and maintained a DMR is, by CML treatment guidelines and CML pregnancy recommendations, the best-suited candidate for therapy interruption [16]

Conclusion

Planned and unplanned pregnancies associated with Myeloproliferative Neoplasms request collaboration between hematologists, gynecologist-obstetrician, neonatologists, and geneticists. A good pregnancy outcome in Myeloproliferative Neoplasms requires an individual therapeutic approach.

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HYPEREOSINOPHILIC SYNDROME – DIAGNOSTIC AND TREATMENT APPROACH

HIPEREOZINOFILNI SINDROM – DIJAGNOZA I TERAPIJSKI PRISTUP

Danijela LEKOVIĆ

Summary

Hypereosinophilic syndrome is defined as a peripheral blood eosinophil count ≥ 1.5 × 10^9/L associated with tissue or organ damage. Eosinophilic disorders represent a group of pathological conditions with heterogeneous pathophysiology, clinical presentation and prognosis. The disease prognosis is based on identifying the subtype and mechanism of eosinophilia. It is important to assess the degree of organ damage based on diagnostics that is directed upon symptoms and signs. After exclusion of secondary causes of eosinophilia, in 2016, the World Health Organization endorsed an assessment towards a molecular classification scheme of disease subtypes named clonal or primary eosinophilias. Diagnostic evaluation of primary eosinophilia relies on a combination of morphologic review of the blood and marrow, standard cytogenetics, fluorescence *in situ* hybridization, flow immunophenotyping, and a T-cell clonality assessment to detect histopathologic or clonal evidence for an acute or chronic myeloid/lymphoid neoplasm. The goal of the therapy is to reduce eosinophil-mediated organ damage. Depending of cause of eosinophilia therapeutic implications range from a ″watch and wait″ to the implementation of allogeneic hematopoietic stem cell transplantation.

Key words: Hypereosinophilic Syndrome; Diagnosis; Therapeutics; Eosinophilia; Signs and Symptoms; Prognosis

Sažetak

Definicija hipereozinofilnog sindroma podrazumeva broj eozinofila u perifernoj krvi ≥ 1,5 × 10^9/L koja je udružena sa postojanjem oštećenja tkiva ili organa. Eozinofilna oboljenja predstavljaju grupu patoloških stanja sa heterogenom patofiziologijom, kliničkom slikom i prognozom. Prognoza bolesti je zavisna od uzroka i mehanizma eozinofilije. Važno je proceniti stepen oštećenja organa na osnovu dijagnostike koja je usmerena prema simptomima i znacima. Nakon isključivanja sekundarnih uzroka eozinofilije, Svetska zdravstvena organizacija 2016. podržava procenu u pravcu molekularne klasifikacije podtipova bolesti pod nazivom klonalne ili primarne eozinofilije. Dijagnostička procena primarne eozinofilije se oslanja na kombinaciju morfološkog pregleda krvi i srži, konvencionalne citogenetike, fluorescentne *in situ* hibridizacije, imunofenotipizacije i procene klonalnosti T-ćelija da bi se otkrili histopatološki ili klonalni dokazi za akutnu ili hroničnu mijeloidnu ili limfoidnu neoplazmu. Cilj terapije je da se smanji oštećenje organa posredovano eozinofilima. U zavisnosti od uzroka eozinofilije, terapeutske implikacije se kreću od režima *watch and wait* do implementacije alogene transplantacije hematopoetskih matičnih ćelija.

Ključne reči: hipereozinofilni sindrom; dijagnoza; terapija; eozinofilija; znaci i simptomi; prognoza

Introduction

Eosinophilic disorders represent a group of pathologic conditions with a highly heterogeneous pathophysiology, clinical presentation and prognosis ranging from asymptomatic to severe with potential for end-organ dysfunction and fatal outcome [1]. Interest for this type of disorders has recently increased with consistent progress in understanding of molecular mechanisms which led to the refining of diagnostic criteria, classification and up-to-date treatment approaches.

The initial concept of hypereosinophilic syndrome (HES) was firstly described by Hardy and Anderson in 1968 as a severe persistent blood eosinophilia with an unknown cause, which leads to multiorgan involvement and a fatal outcome [2]. The first diagnostic criteria of primary or idiopathic HES were published in 1975 by Chusid as following: a persistent blood absolute eosinophil count over 1500/mm^3 for a duration of more than 6 months, with evidence of tissue and organ damage, without any identifiable cause of eosinophilia [3]. Since then, it was known that a subgroup of patients with HES had clinical and hematological features of myeloproliferative disease such as splenomegaly, anemia and myelofibrosis.

The reference value of an absolute eosinophil count (AEC) in peripheral blood is considered to be 350 – 500/mm^3 and a percentage of 3–5% of the total number of white blood cells. The term eosinophilia is recommended for a small increase in AEC from 500 – 1500/mm^3. Hypereosinophilia (HE) is defined based on AEC greater than 1500/mm^3 on two consecutive occasions, persistent for at least 1 month (rather than 6 months, as previously considered in the definition of HES) [4]. According to severity-based classification, HE is divided into mild (AEC from the upper limit of normal to 1.5 × 10^9/L), moderate (AEC 1500 – 5000/mm^3) and severe (AEC > 5000/mm^3). Tissue HE is defined by the following: the percentage of eosinophils in bone marrow section exceeds 20% of all nucleated cells and/or extensive tissue infiltration by eosinophils

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Abbreviations

HES – hypereosinophilic syndrome
AEC – absolute eosinophil count
HE – Hypereosinophilia
WHO – World Health Organization
TCR – T-cell receptor
ICOG-EO – International Working Group on Eosinophil Disorders
CEL, NOS – chronic eosinophiemialeukaemia, not otherwise specified
IL-5 – interleukin-5

based on a pathologist report and/or marked deposition of eosinophil granule proteins (in the absence or presence of major tissue infltration by eosinophils).

Since the early 2000s, the definition and diagnostic criteria of different subgroups of hypereosinophilic disorders have been revised by many panels of experts, mainly hematologists. Classification of myeloid neoplasms, including primary HES was proposed by the World Health Organization (WHO) in 2008 and later revised in 2016 [5]. A more complex classification of eosinophilic disorders was proposed in 2011 by a larger international and multidisciplinary panel of experts (International Working Group on Eosinophil Disorders (ICOG-EO)), who agreed on terminology and diagnostic criteria of various forms of HE and HES [6]. This classification retained the criteria regarding the level of blood eosinophilia, but changed the duration period from 6 months to 1 month, adding tissue eosinophilia, and including some particular conditions, such as asymptomatic, associated and overlap forms of eosinophilia. HE of undetermined significance is characterized by peripheral blood HE and no clinical symptoms and/or proof of organ dysfunction. Overlap HE syndromes are conditions that associate single organ-restricted eosinophilia, which may be preceded or accompanied by peripheral eosinophilia, such as eosinophilic gastrointestinal disorders, eosinophilic esophagitis, eosinophilic pneumonia, and eosinophilia-myalgia syndrome. Associated HE includes various subtypes of HES associated with other conditions, known as causes of reactive HE, such as systemic mastocytosis, infections, inflammatory bowel disease, systemic vasculitis or other autoimmune diseases.

Organ damage may occur in HES independently of the underlying subtype. The most common presenting signs and symptoms are weakness and fatigue (26%), cough (24% neadyspnoea (16%), myalgias or angioedema (14%), rash or fever (12%), and rhinitis (10%) [7]. Commonest laboratory finding in HES sisleucocytosis ($\geq$ 20–30×10^9/L) with peripheral eosinophilia in the range of 30–70% [7, 8]. Other hematologic findings include peripheral blood or bone marrow neutrophilia, basophilia, myeloid immaturity and both mature and immature eosinophils with varying degrees of dysplasia [9]. Anemia has been identified in 50% of patients, thrombocytopenia was more common than thrombocytosis (30% vs. 15%), and bone marrow eosinophilia ranged from 7 – 57% (average 33%) [10]. Specific findings in the bone marrow are Charcot-Leyden crystals (a hallmark of eosinophilic inflammation containing the protein galectin-l0) and, in some cases, increased blasts and bone marrow fibrosis [9, 10].

Almost any organ can be damaged as a result of HES, but the most common are skin (69%), then pulmonary (44%) and gastrointestinal (38%) manifestations. Cardiac damage was identified in 20% of patients [11]. Progressive heart failure is typical example of eosinophil-mediated organ damage. It involves a multistep pathophysiological process involving eosinophil infiltration of cardiac tissue as well as release of toxic mediators from eosinophils [11]. Endocardial damage can lead to the formation of thrombi and an increased embolic risk. In the later fibrotic stage, fibrous thickening of the endocardial lining can cause a restrictive cardiomyopathy. Valvular insufficiency is a consequence of mural endocardial thrombosis and fibrosis of the mitral or tricuspid valves.

Firstly, it is advised to exclude a cause of secondary eosinophilia which is a common consequence of infection, especially tissue-invasive parasites

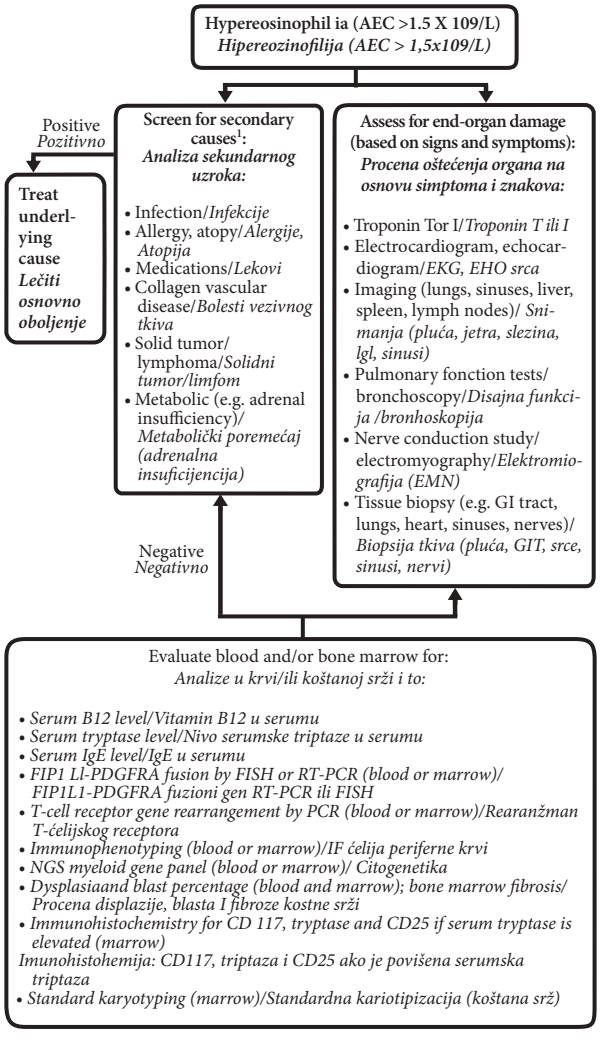

Figure 1. Diagnostic algorithm of eosinophilic disorders
Slika 1. *Dijagnostički algoritam eozinofilnih oboljenja*

(Figure 1) [12]. Crucial for identification of infectious tetiology is travel history, repeated ova and parasite testing, stool culture and antibody testing for specific parasites (e.g. strongyloides). In the differential diagnosis of a secondary cause of eosinophilia, the following should be considered: allergy, drug reactions, collagen-vascular disease (eg., eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), granulomatosis with polyangiitis (Wegener's), systemic lupus erythematosus), pulmonary eosinophilic diseases (eg., idiopathic acute or chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis), allergic gastroenteritis (with associated peripheral eosinophilia), and metabolic conditions such as adrenal insufficiency [11–13]. Non-myeloid malignancies may be associated with secondary eosinophilia which results from the production of cytokines, such as IL-3, IL-5, and GM-CSF, which promote eosinophil differentiation and survival. For example, these cytokines may be elaborated from malignant cells in T-cell lymphomas, Hodgkin lymphoma, and acute lymphoblastic leukemia. Rare conditions associated with eosinophilia include familial eosinophilia with unknown genetic basis including hyper IgE syndrome, Omenn syndrome, episodic angioedema and eosinophilia (Gleich's syndrome), and eosinophilia-myalgia syndrome (.g., possibly related to tryptophan ingestion). Elevated immunoglobulin E levels is a non-specific finding that is mostly seen in reactive conditions (infectious, allergic, vasculitis and lymphocyte-variant HES), whereas its elevation is variable in patients with clonal HES/CEL, NOS.

Assessment of the degree of organ damage based on diagnostics that is directed towards symptoms and signs **(Figure 1)**. Additional laboratory testing, imaging tests, electrocardiogram and echocardiography, CT scan of the chest, abdomen and pelvis) are guided by presymptoms, symptoms and findings on physical examination. For the characterization of lung involvement in eosinophilic lung diseases, the following analyses should be considered: pulmonary function testing, bronchoscopy and serologic tests (.g., aspergillus IgE to evaluate for allergic bronchopulmonary aspergillosis).

After excluding the cause of secondary (reactive) eosinophilia, the diagnostic evaluation of primary eosinophilia is based on cytomorphological examination of blood and marrow, analysis of conventional cytogenetics, immunophenotyping of cells, analysis of T cell receptor (TCR) and FISH analysis of gene rearrangement in order to detect histopathological or clonal signs of acute or chronic myelo/lymphoid neoplasms **(Table 1)** [9, 10].

In addition to the diseases listed in **Table 1**, there is an MPN subtype named "chronic eosinophilic leukemia, not otherwise specified" (CEL, NOS) [5, 14]. A negative screen for eosinophilias related to the aforementioned fusion tyrosine kinases should prompt consideration of a diagnosis of CEL, NOS when there is cytogenetic, molecular and/or morphologic evidence of an eosinophilic myeloid malignancy that is otherwise not classifiable. CEL, NOS may be distinguished from

Table 1. Revised 2016 WHO classification of myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDGFRB, FGFR1 or PCM1-JAK2
Tabela 1. *Revidirana SZO klasifikacija mijeloidnih/limfoidnih neoplazmi sa eozonofilijom i rearanžmanom PDGFRA, PDGFRB, FGFR1 ili PCM1-JAK2*

Gen/*Gen*	Diagnostic criteria/*Dijagnostički kriterijumi*
PDGFRA	MPNa with eosinophilia associated with FIP1L1-PDGFRA A myeloid or lymphoid neoplasm, usually with prominent eosinophilia and Presence of *FIP1L1*-PDGFRA fusion gene or a variant fusion gene with PDGFRA rearrangement/*Mijeloidna/limfoidna neoplazma sa izraženom eozinofilijom + FIP1L1-PDGFRA fuzioni gena ili rearanžan PDGFRA*
PDGFRB	Myeloid/lymphoid neoplasms associated with ETV6-PDGFRB fusion gene or other rearrangement of PDGFRB Myeloid or lymphoid neoplasm, often with prominent eosinophilia and sometimes with neutrophilia or monocytosis and Presence of t(5;12)(q31~q33;p12) or a variant translocationd or demonstration of an ETV6-PDGFRB fusion gene or rearrangement of *PDGFRB/Mijeloidna/limfoidna neoplazma sa eozinofilijom, ponekad sa neutrofilijom ili monocitozom + t(5;12)(k31~k33; p12) ili varijante translokacije koje dovode ETV6-PDGFRB fuzionog gena ili rearanžmana PDGFRB*
FGFR1	MPN or acute leukemia associated with FGFR1 rearrangement MPN or MDS/MPN with prominent eosinophilia, and sometimes with neutrophilia or monocytosis or Acute myeloid leukemia or precursor T-cell or precursor B-cell lymphoblastic leukemia/lymphoma or mixed phenotype acute leukemia (usually associated with peripheral blood or BM eosinophilia) and Presence of t(8;13)(p11;q12) or a variant translocation leading to FGFR1 rearrangement demonstrated in myeloid cells, lymphoblasts, or both/*MPN ili MPN/MDS sa eozinofilijom, ponekad sa neutrofilijom ili monocitozom ili AML ili prekursor T-ćelija ili prekursor B-ćelijska limfoblastna leukemija/limfom ili mešoviti fenotip AL (povezana sa eozinofilijom u perifernoj krvi ili koštanoj srži) + t(8;13)(p11;k12) ili varijante translokacije koje dovode do rearanžmana FGFR1*
PCM1-JAK2	Myeloid/lymphoid neoplasms with PCM1-JAK2 A myeloid or lymphoid neoplasm, often with prominent eosinophilia and Presence of t(8;9)(p22;p24.1) or a variant translocation leading to JAK2 rearrangemente *Mijeloidna/limfoidna neoplazma, sa eozinofilijom + t(8;9)(p22;p24.1) ili rearanžman JAK2 gena*

HES by the presence of a non-specific clonal cytogenetic or molecular abnormality or increased blast cells (>2% in the peripheral blood or > 5% in the bone marrow, but <20% blasts in both compartments) [10].

Lymphocytic variant of HES is an entity with hypereosinophilia due to overproduction of eosinophilopoietic cytokines, by a clonal population of activated T-lymphocytes [5]. The mechanism can be considered both clonal and reactive, since eosinophilia is reactive to the eosinophilopoietic growth factors, mainly IL-5, produced by the abnormal population of T-lymphocytes, with an atypical pattern of surface markers and a T helper cell type 2.

After excluding secondary causes of eosinophilia, according to the WHO classification from 2016, it is advised to analyze the existence of myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of *PDGFRA, PDGFRB, FGFR1* or myeloid neoplasms - chronic eosinophilic leukemia (CEL) [9, 10, 14]. The most common molecular aberration is the *FIP1L1-PDGFRA* fusion gene, identified by *RT-PCR* or *FISH* analysis. In addition to eosinophilia, clinical, laboratory and morphological characteristics in patients with PDGFRA/B rearrangement can have: 1. Elevated tryptase, vitamin B12 and LDH in serum; 2. Occurrence of cytopenia and monocytosis; 3. Hepatosplenomegaly and lymphadenopathy; 4. Presence of bone marrow fibrosis and an increased number of diffusely distributed CD25+ mast cells bone marrow [15, 16]. After ruling out *PDGFRA* gene rearrangement an evaluation for other clonal eosinophilia is suggested including *FISH* analysis of *PDFRB* rearrangement if it is available as well as analysis of morphological signs of myeloid/lymphoid neoplasms associated with eosinophilia with or without cytogenetic aberrations and/or lymphocytic variants HE. In the case of cytogenetically observed translocation of the chromosome region, it is advised to establish a mandatory confirmation by *FISH* analysis, which is also

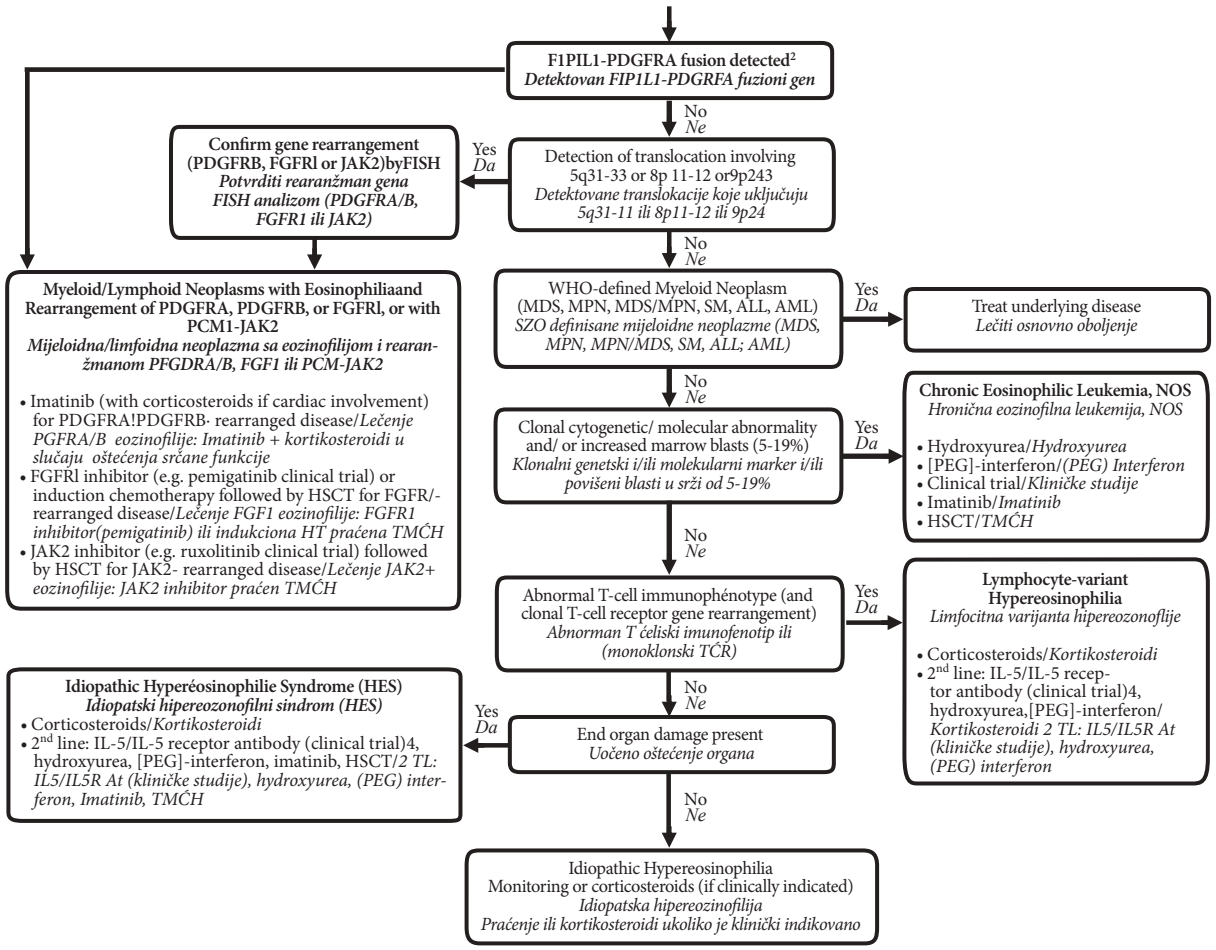

Figure 3. Treatment algorithm based on the revised WHO 2016 classification of eosinophilic disorders
Slika 3. *Algoritam lečenja baziran na SZO 2016 klasifikaciji eozinofilnih oboljenja*
FISH - Fluorescence in situ hybridization, HSCT - Hematopoietic Stem Cell Transplantation, At - Antibody, MDS - Myelodysplastic syndromes, MPNs - Myeloproliferative neoplasms, AML - Acute Myeloblastic Leukemia, ALL - Acute lymphocytic leukemia
FISH – fluorescentna in situ *hibridizacija; TMĆH – transplantacija matičnih ćelija hematopoeze; At – antitelo; MDS – mijelodisplastični sindrom; MPN – mijeloproliferativna neoplazma; AML – akutna mijeloblastna leukemija; ALL – akutna limfoblastna leukemija*
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required to assess the response to therapy [15, 16]. Unlike patients with *FIP1L1-PDGFRA* rearrangement, patients with *PDGFRB* or *FGFR1* rearrangement can be diagnosed without eosinophilia in peripheral blood [16, 17]. Diseases with *PCM1-JAK2* rearrangement have been added to the WHO classification of eosinophilic diseases as a provisional entity. A fusion gene with *FLT3,* most commonly *ETV6-FLT3*, is usually associated with MPN and/or acute lymphoblastic T-cell leukemia/lymphoma with eosinophilia, although this entity has not yet been formally added to the WHO classification.

The prognosis of the disease depends on the type of eosinophilia. Poor prognostic parameters osisleucocytosis >100 x 10^9/L, impairment of cardiac function and refractoriness to corticosteroids.

The goal of treatment is to reduce eosinophil-mediated organ damage. For patients with milder forms of eosinophilia (AEC < 1.5×10^9/L) without symptoms or signs of organ damage, a ″watch and wait″ regimen with careful monitoring is advised. When a *PDGFRA* or *PDGFRB* gene rearrangement is identified, the therapy of choice is imatinib **(Figure 3)** [15, 16]. Corticosteroids are the first line treatment for patients with HES and the lymphocytic variant of HE. Hydroxyurea and interferon have shown therapeutic efficacy in patients who have resistance to corticosteroid treatment. Mepolizumab, a monoclonal antibody antagonist of interleukin-5 (IL-5), was recently approved by the US Food and Drug Administration for patients with idiopathic HES [17]. The use of the IL-5 receptor antibody benralizumab, as well as other targeted therapies such as JAK2 (ruxolitinib) and FGFR1 (pemigatinib) inhibitors are still being investigated in clinical trials. In patients with myeloid neoplasm associated with *FGFR1* rearrangement, allogeneic stem cell transplantation remains an important treatment option [18–20].

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CLINICAL SIGNIFICANCE OF BLEEDING SCORING SYSTEMS

KLINIČKI ZNAČAJ SISTEMA BODOVANJA ZA KRVARENJA

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Summary

Introduction. Bleeding scoring systems are used for predicting the risk of a specific primary outcome in a specific population.In this article, we present scoring systems frequently used in internist practice. **Bleeding scoring systems for acute upper gastrointestinal bleeding**. Are divided into clinical, endoscopic,and combined scoring systems, according to the type of variables included. Besides traditional systems (Rockall, Glasgow-Blatchford), we primarily present clinical scoring systems used for upper gastrointestinal bleeding. **Bleeding scoring systems for patients with atrial fibrillation on anticoagulant therapy.** We refer to the most significant scoring systems - ATRIA, HAS-BLED, and ORBIT. Since the prognostic performance of the existing scoring systems is less than satisfactory, medical organizations suggest they should be used for recognizing patients who deserve close monitoring during anticoagulant therapy. **Bleeding scoring systems for patients with acute myocardial infarction treated with percutaneous coronary intervention**. ACUITY-HORIZONS was proven to be superior to other scoring systems in predicting bleeding within 30 days in patients with a myocardial infarction treated with transradial percutaneous coronary intervention. **Bleeding scoring systems for bleeding disorders**. Scoring systems enable the detection of bleeding disorders before performing laboratory tests. Considering the frequency severity of symptoms, newer systems have been developed for the pediatric population and self-testing by modifying the original scoring system. **Conclusion**. Although medical organizations encourage the implementation of scoring systems in clinical practice, the development of newer and enhanced existing systems requires a comprehensive approach and critical analysis of the existing systems.

Key words: Hemorrhage; Risk Assessment; Prognosis; Anticoagulants; Atrial Fibrillation; Percutaneous Coronary Intervention; Gastrointestinal Hemorrhage

Sažetak

Uvod. Sistemi bodovanja za krvarenja koriste se za predviđanje rizika od određenog primarnog ishoda u određenoj populaciji pacijenata. U radu predstavljamo sisteme bodovanja za krvarenja koji se najčešće koriste u internističkoj praksi. **Sistemi bodovanja za krvarenja iz proksimalnog gastrointestinalnog trakta.** Dele se prema vrsti sadržanih varijabli na kliničke, endoskopske i kombinovane sisteme bodovanja. Pored tradicionalnih sistema bodovanja (*Rockall* i *Glasgow-Blatchford*) koji se primenjuju kod krvarenja iz gornjih partija probavnog trakta, navodimo i druge prevashodno kliničke sisteme bodovanja. **Sistemi bodovanja za krvarenja kod pacijenata sa atrijalnom fibrilacijom na antikoagulantnoj terapiji.** Po značaju se izdvajaju sistemi ATRIA, HAS-BLED i ORBIT. Zbog ograničenog prognostičkog uspeha postojećih sistema bodovanja stručna udruženja preporučuju da se oni koriste za otkrivanje pacijenata koji zahtevaju češće kontrole i obazrivije sprovođenje antikoagulatne terapije. **Sistemi bodovanja za krvarenja kod pacijenata sa srčanim udarom lečenih perkutanom koronarnom intervencijom**. ACUITY-HORIZONS se pokazao pouzdanijim u odnosu na druge sisteme u predviđanju nastanka krvarenja u prvih 30 dana od srčanog udara kod pacijenata lečenih perkutanom koronarnom intervencijom transradijalnim pristupom. **Sistemi bodovanja za krvarenja kod pacijenata sa krvarećim poremećajima**. Pomoću sistema bodovanja moguće je otkriti postojanje krvarećeg poremećaja pre sprovođenja laboratorijskih testova. Modifikacijama izvornih sistema načinjeni su sistemi koji pored težine u obzir uzimaju i učestalost simptoma, kao i sistemi prilagođeni za pedijatrijsku populaciju i samotestiranje. **Zaključak**. Iako medicinska stručna udruženja ohrabruju upotrebu postojećih sistema bodovanja za krvarenja u praksi, razvoj novih i poboljšanje postojećih sistema bodovanja zahteva sveobuhvatan pristup uz kritičku analizu postojećih sistema.

Ključne reči: krvarenje; procena rizika; prognoza; antikoagulantna terapija; atrijalna fibrilacija; perkutana koronarna intervencija; gastrointestinalno krvarenje

Introduction

Bleeding Scoring Systems (BSSs) are clinical tools used for quantitative assessment and predicting the risk of a specific primary outcome in a specific patient population [1, 2].

There are various primary outcomes, such as the risk of bleeding occurrence, risk of rebleeding, bleeding mortality risk, or the need for therapeutic intervention, depending on the BSS used [2, 3].

Some BSSs are known as Bleeding Assessment Tools (BATs) which are used to determine the likli-

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Abbreviations

BSS – Bleeding Scoring System
BAT – Bleeding Assessment Tool
BBS – Baylor Bleeding Score
CSMCPI – Cedars-Sinai Medical Center Predictive Index
PNED – progetto nazionale emorragia digestiva
GBS – Glasgow-Blatchford score
AIMS65 – Albumin < 3.0 g/dl, International normalized ratio > 1.5, altered Mental status, Systolic blood pressure < 90 mmHg, age above 65 years
RS – Rockall score
AUGB – acute upper gastrointestinal bleeding
Mt – mortality
Hgb – haemoglobin
Hct – haematocrit
INR – international normalized ratio
AF – atrial fibrillation
ATRIA – Anticoagulation and Risk Factors in Atrial Fibrillation
HAS-BLED – Hypertension, Abnormalities of liver and/or kidney abnormalities, Stroke history, Bleeding history, Labile INR, Elderly, Drugs and/or alcohol use
NSAID – non-steroidal anti-inflammatory drug
ORBIT – Older age, Reduced hemoglobin/hematocrit/ anemia, Bleeding history, Insufficient renal function, Treatment with antiplatelet drugs
ORBIT-AF – Outcomes Registry for Better Informed Treatment of Atrial Fibrillation
ROCKET-AF – Riveroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation
ESC – European Society of Cardiology
AHA – American Heart Association
ACC – American College of Cardiology
HRS – Heart Rhythm Society
AMI – acute myocardial infarction
PCI – percutaneous coronary intervention
TR – transradial
CRUSADE – Can Rapid risk stratification of Unstable angina patients Suppress Adverse outcomes with Early implementation of the ACC/AHA Guidelines
STEMI – acute myocardial infarction with ST segment elevation
ACS – acute coronary syndrome
ACTION – Acute Coronary Treatment and Intervention Out comes Network
SBP – systolic blood pressure
ECG – electrocardiogram
ACUITY-HORIZONS – Acute Catheterization and Urgent Intervention Triage strategY- Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction
VWD1 – von Willebrand disease type 1
ISTH – International Society on Thrombosis and Haemostasis
PBQ – Pediatric Bleeding Questionnaire
VWD – von Willebrand disease
MCMDM-1 VWD – The European Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD
MCMDM-1 VWD BQ – The European Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD Bleeding Questionnaire
BS – bleeding score
ISTH-BAT – International Society on Thrombosis and Hemostasis Bleeding Assessment Tool
Self-BAT – self-administered Bleeding Assessment Tool

hood of a patient having certain bleeding disorders before further laboratory investigations [4].

When using a specific BSS, one must know the primary outcome variable that it was developed for [2].

An optimal BSS should be simple (minimal number of variables, short administration time, easy to use and remember, some are used for self-testing), objective (containing quantitative variables, minimal subjectivity in symptom variable interpretation), available and accessible for use, highly sensitive (true positives/all positive tests) and highly specific (true negatives/all negative tests), as well as validated (proven to be reliable on a real-life population) and reliable in predicting a specific primary outcome in a particular population [2, 4–6].

Since no ideal BSS was developed for any bleeding issue, many prediction tools have been developed to address a certain medical bleeding issue. The dilemma regarding the optimal BSS for determining a specific primary outcome has been a subject of recent medical research.

In this review article, we have presented a short review of the most frequently used BSSs in internist practice.

BSSs used for acute upper gastrointestinal bleeding

BSSs used for acute bleeding above the level of the suspensory muscle of the duodenum (the ligament of Treitz) are divided based on whether they require only clinical variables, or only endoscopic variables, or both, into clinical BSSs (clinical Rockall score, Glasgow-Blatchford score, AIMS65, T-score), endoscopic BSSs (Forrest), and combined BSSs (full Rockall score, BBS, CSMCPI, PNED) [2, 7, 8].

The Glasgow-Blatchford score (GBS) and the Rockall score (RS) are the most cited BSSs for acute upper gastrointestinal bleeding (AUGB) [2, 7].

Rockall score (with its variations - admission/ clinical RS, endoscopic RS, full RS) is a BSS used primarily for predicting AUGB mortality, as well as the risk of rebleeding **(Table 1)** [2, 7]. This BSS was developed in 1996. by Rockall et al., and it includes the following variables: age, comorbidities, hemodynamic status, endoscopic findings, and the stigmata of recent hemorrhage (adherent clot, blood in upper gastrointestinal tract, visible vessel/spurting vessel), whereas the clinical RS includes only three variables: age, comorbidities and the hemodynamic status [2]. The values of RS ≤ 2 stratify the patient into the group with low risk from rebleeding and death due to AUGB; therefore, these patients can be safely managed in outpatient setting since Rockall et al. reported the rates of rebleeding and mortality in the low-risk patient group to be 4.3% and

Table 1. Rockall score*
Tabela 1. *Rockall skor**

Score Variable *Varijabla skora*	0	1	2	
Age/*Starost*	< 60 years/*< 60 godina*	60–79 years/*60–79 godina*	≥ 80 years/*≥ 80 godina*	
Shock/*Šok*	No shock, systolic BP ≥ 100, pulse < 100 *Bez šoka, sistolni KP ≥ 100, puls < 100*	Tachycardia, systolic BP ≥ 100, pulse ≥ 100 *Tahikardija, sistolni KP ≥ 100, puls ≥ 100*	Hypotension, systolic BP < 100 *Hipotenzija, sistolni KP < 100*	
Comorbidity *Komorbiditeti*	No major comorbidity *Bez značajnih komorbiditeta*		Cardiac failure, ischemic heart disease, any major comorbidity *Srčana slabost, ishemijska bolest srca, bilo koji značajniji komorbiditet*	Renal failure, liver failure, disseminated malignancy *Bubrežna slabost, hepatična insuficijencija, diseminovani malignitet*
Diagnosis *Dijagnoza*	Mallory-Weiss tear, no lesion identified and no SRH *Malori-Vajsov rascep, bez identifikovane lezije i bez SSK*	All other diagnoses *Sve ostale dijagnoze*	Malignancy of UGI tract *Malignitet GGI trakta*	
Major SRH *Velike SSK*	None or dark spots only *Bez mrlje ili samo tamna mrlja*		Blood in UGI tract, adherent clot, visible or spurting vessel *Krv u GGI traktu, adherirani ugrušak, krvni sud koji se uočava ili prska*	

Admission score: Sum of age, shock and comorbidity; full score: Sum of age, shock, comorbidity, diagnosis and major SRH; BP: Blood pressure (measured in mmHg); UGI: Upper gastrointestinal; SRH: Stigmata of recent haemorrhage. *(Modified from Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: Who, when and why? World J Gastrointest Pathophysiol [Internet]. 2016 Feb [cited 2022 Aug 9]; 7(1):[86-96]. Available from: https://www.wjgnet.com/2150-5330/full/v7/i1/86.htm)

*Prijemni skor: Suma bodova za varijable starosti, šoka i komorbiditeta; kompletan skor: suma bodova za varijable starosti, šoka i komorbiditeta, diagnoza i velike SSK; KP: krvni pritisak (izražen u mmHg); GGI: gornji gastrointestinalni; SSK: stigma skorašnjeg krvarenja. *(Modifikovano prema Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: Who, when and why? World J Gastrointest Pathophysiol [Internet]. 2016 Feb [cited 2022 Aug 9]; 7(1):[86-96]. Available from: https://www.wjgnet.com/2150-5330/full/v7/i1/86.htm)*

0.1% respectively [2, 9]. However, the values of RS > 2 classify the patient into a high-risk group, thus requiring hospitalization and esophagogastroduodenoscopy [2, 9]. According to the results of Church and Palmer, unfavorable outcome is expected particularly in patients with RS ≥ 8, while the results of Tham et al. suggest that patients with a clinical RS = 0 do not require blood transfusions, and they can be safely managed in outpatient setting [10, 11]. Phang et al. have shown that clinical RS can be used for determining the proper environment into which AUGB patients ought to be admitted, suggesting that patients with clinical RS ≥ 4 (high-risk; Mt = 22.4%) ought to be admitted into an intensive care facility, while patients with clinical RS < 4 (low-risk; Mt = 3.2%) ought to be admitted into a general ward [2, 12].

Glasgow-Blatchford score (GBS) is a clinical BSS used for predicting the need for therapeutic intervention (blood transfusions, endoscopic treatment, surgery) in AUGB patients **(Table 2)** [2, 7]. This BSS includes 8 variables (melaena, syncope, heart rate, systolic blood pressure, hemoglobin (Hgb), blood urea nitrogen, liver disease and heart failure), whereas in the modified GBS syncope and blood urea nitrogen variables are excluded [2, 7]. Although GBS is more sensitive than RS in identifying AUGB patients that are in need of therapeutic intervention, this BSS has low specificity and, therefore low positive predictive value overestimating the risk of an unfavorable outcome [2]. The significance of GBS is in predicting the need for hospitalization (and therapeutic intervention) in patients with AUGB because patients with GBS = 0 can be safely managed in the outpatient setting, thus not requiring hospitalization [2, 8]. The higher the GBS, the greater the need for therapeutic intervention in the hospital setting [2].

AIMS65 is a clinical BSS for AUGB which includes 5 variables: A - albumin < 3.0 g/dl, I - international normalised ratio (INR) > 1.5, M - altered mental status, S - systolic blood pressure < 90 mmHg, 65 - age above 65 years, each awarded one point [2, 13]. If a patient scores AIMS65 > 2, the risk of AUGB mortality is high, whereby AIMS65 can also be used to estimate the cost

Table 2. Glasgow-Blatchford score*
Tabela 2. *Glasgow-Blatchford skor**

Variable/*Varijabla*	Score/*Skor*
Blood urea (mmol/L)/*Urea (mmol/L)*	
6.5-8	2
8-10	3
10-25	4
> 25	6
Hgb (g/L) for men/*Hgb (g/L) kod muškaraca*	
120-130	1
100-120	3
< 100	6
Hgb (g/L) for women/*Hgb (g/L) kod žena*	
100-120	1
< 100	6
Systolic blood pressure (mmHg)/*Sistolni krvni pritisak (mmHg)*	
100-109	1
90-99	2
< 90	3
Pulse ≥ 100/min/*Puls ≥ 100/min*	1
History and comorbidities/*Anamneza i komorbiditeti*	
Melaena/*Melena*	1
Syncope/*Sinkopa*	2
Hepatic disease[1]/*Bolest jetre*[1]	2
Cardiac failure[2]/*Srčana slabost*[2]	2

[1]Known history or clinical and laboratory evidence of chronic or acute liver disease;
[2]Known history or clinical and echocardiographic evidence of cardiac failure.
*(Modified from Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: Who, when and why? World J Gastrointest Pathophysiol [Internet]. 2016 Feb [cited 2022 Aug 9]; 7(1):[86-96]. Available from: https://www.wjgnet.com/2150-5330/full/v7/i1/86.htm)
[1]Anamnestički podatak, klinički ili laboratorijski dokazi o postojanju hronične ili akutne bolesti jetre;
[2]Anamnestički podatak ili postojanje kliničkih ili ehokardiografskih dokaza o postojanju srčane slabosti.
**(Modifikovano prema Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: Who, when and why? World J Gastrointest Pathophysiol [Internet]. 2016 Feb [cited 2022 Aug 9]; 7(1):[86-96]. Available from: https://www.wjgnet.com/2150-5330/full/v7/i1/86.htm)*

and duration of hospitalization [2, 13]. Although AIMS65 is inferior to GBS in predicting the need for blood transfusions (GBS, unlike AIMS65, includes Hgb), it is superior to GBS in predicting in-hospital mortality [2]. Masaoka et al. suggested an algorithm for predicting AUGB mortality by which AIMS65 should be calculated in patients with GBS ≤ 2 (low-risk) [2, 14].

T-score is a clinical BSS which includes 4 variables: Hgb (≤ 8 g/dL; 9-10 g/dL; > 10 g/dL), systolic blood pressure (< 90 mmHg; 90-110 mmHg; > 110 mmHg), pulse (< 90/min; 90-110/min; > 110/min), general conditions (poor, intermediate, good) [2, 15]. T-score ≤ 6 predicts the existence of high-risk endoscopic stigmata, a greater risk of rebleeding from the upper gastrointestinal tract, as well as greater AUGB mortality risk [2, 15, 16].

Cedars-Sinai Medical Center Predictive Index (CSMCPI) was developed by Hay et al., whereby this BSS predicts the length of hospitalization based on 4 variables (symptoms at the time of AUGB, number of comorbidities, hemodynamic instability, endoscopic findings) **(Table 3)** [2, 17]. The value of CSMCPI = 3 stratifies a patient into a group with low rebleeding risk and low AUGB mortality risk; thus these patients can be safely discharged from the hospital within the first 24h and continue their treatment in an outpatient setting [17]. According to the results of Benedeto-Stojanov et al. CSMCPI was shown to be superior to RS and GBS in means of sensitivity, specificity, positive and negative predictive values, highest positive and lowest negative likelihood ratio, as well as higher odds of mortality [7].

BSSs used for assessment of bleeding risk in patients with atrial fibrillation on anticoagulant therapy

During atrial fibrillation (AF), turbulent blood flow exists in the heart (especially in the auriculae), precipitating the formation of a thrombus, which fragments itself, and its fragments (emboli) spread through circulation, creating an obstruction of the lumina of arterial branches which participate in the perfusion of the brain, causing ischemic stroke [18, 19].

Table 3. Cedars-Sinai Medical Center Predictive Index*
Tabela 3. *Prediktivni indeks Cedars-Sinai medicinskog centra**

Score *Skor*	EGD findings[1]/*EGD nalazi[1]*	Time[2] *Vreme[2]*	Hemodynamics *Hemodinamika*	Comorbidities *Komorbiditeti*
0	Ulcer without SHR, non-bleeding Mallory-Weiss tear, Erosive disease, normal EGD/*Ulkus bez SSK, Mallory-Weiss rascep koji ne krvari, Erozivna bolest, normalan EGD nalaz*	> 48 h	Stable/*Stabilna*	≤ 1
1	Ulcer with flat spot or clot, an erosive disease with SHR, angiodysplasia/*Ulkus sa ravnom mrljom ili ugruškom, erozivna bolest sa SSK, angiodisplazija*	< 48 h	Intermediate *Srednja*	2
2	Ulcer with non-bleeding visible vessel or SHR *Ulkus sa SSK ili vidljivim krvnim sudom koji ne krvari*	In hospital *U bolnici*	Unstable *Nestabilna*	3
3				≥ 4
4	Persistent hemorrhage, varices UGI cancer *Persistentno krvarenje, variksi Malignitet GGI*			

[1]Score for endoscopic findings was reduced by 1 point if effective endoscopic therapy was applied (not applicable to varices or cancer); [2]Time from the onset of symptoms to hospitalization; SHR: Stigmata of recent hemorrhage; EGD: Esophagogastroduodenoscopy; UGI: Upper gastrointestinal
*(Modified from Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: Who, when and why? World J Gastrointest Pathophysiol [Internet]. 2016 Feb [cited 2022 Aug 9]; 7(1):[86-96]. Available from: https://www.wjgnet.com/2150-5330/full/v7/i1/86.htm)
[1]Skor za endoskopske nalaze je umanjen za 1 bod u slučaju da je primenjeno efikasno endoskopsko lečenje (ne primenjuje se kod prisustva variksa ili maligniteta); [2]Vreme od pojave simptoma do hospitalizacije; SSK: stigma skorašnjeg krvarenja; EGD: ezofagogastroduodenoskopija; GGI: gornji gastrointestinalni
**(Modifikovano prema Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: Who, when and why? World J Gastrointest Pathophysiol [Internet]. 2016 Feb [cited 2022 Aug 9]; 7(1):[86-96]. Available from: https://www.wjgnet.com/2150-5330/full/v7/i1/86.htm)*

The administration of anticoagulant therapy in patients with AF inhibits the activity of coagulation factors, thus diminishing the risk of ischemic stroke occurrence, but at the same time, anticoagulant therapy compromises hemostasis; therefore any bleeding can not be stopped spontaneously [18–20]. Factors that increase the bleeding risk in patients with AF treated with anticoagulants are old age, comorbidities (liver disease, kidney disease, malignancy), as well as administered doses of antiplatelet and anticoagulant drugs [18, 19, 21]. Here we will mention ATRIA, HAS-BLED, and ORBIT scoring systems which are used for the assessment of bleeding risk in patients with AF on anticoagulant therapy [18, 19].

ATRIA ("Anticoagulation and Risk Factors in Atrial Fibrillation") is a BSS that includes 5 variables: severe renal disease (3 points), anemia (3 points), age ≥ 75 years (2 points), hypertension (1 point), and prior hemorrhage (1 point) [18, 19, 22].

HAS-BLED is a BSS that includes the following variables: H - hypertenzion, A - abnormalities of liver and/or kidney abnormalities (each awarded 1 point), S - stroke history, B - bleeding history, L - labile INR, E - elderly (> 65 years), D - drugs and/or alcohol use **(Table 4)** [18, 19, 23]. Patients with a HAS-BLED score ≥ 3 (high-risk) deserve regular clinical review and follow-up; however a high HAS-BLED score alone is no reason for stopping oral anticoagulant therapy [24]. By using HAS-BLED, it is possible to identify reversible risk factors for bleeding events during anticoagulant therapy in patients with AF (alcoholism, uncontrolled hypertension, concomitant use of NSAID, labile INR) [18,19].

ORBIT is a BSS that includes 5 variables: O - older age (1 point), R - reduced Hgb/Hct/anemia (2 points), B - bleeding history (2 points), I - insufficient renal function (1 point), and T - treatment with antiplatelet drugs (1 point) **(Table 4)** [18, 19, 23]. The main limitation of ORBIT is that elderly patients often have age-related kidney failure and/or age-related anemia; therefore this overlap may increase the overall ORBIT score [18].

HAS-BLED and ORBIT systems are used for predicting the risk of major bleeding events and mortality in patients with AF on anticoagulant therapy. In contrast the results of c-statistics suggest that these two BSSs are equally significant in predicting the risk of major bleeding events and mortality during treatment with acenocoumarol or direct oral anticoagulants [18]. According to the results of ORBIT-AF and ROCKET-AF trials ORBIT score had broader applicability and was more successful in calibrating bleeding risk compared to ATRIA or HAS-BLED [18, 25]. However, it has been shown that HAS-BLED is superior to ATRIA and ORBIT in assessing the risk of bleeding and mortality in patients with AF treated with warfarin and idraparinux [18, 26, 27]. The main advantages of HAS-BLED are that it is more successful than ORBIT in identifying patients with a low bleeding risk; HAS-BLED is an adequate BSS for patients younger than 75 years with a low bleeding risk, whereas HAS-BLED is more precise in risk stratification in patients with a risk for stroke, who are treated with warfarin / non-warfarin anticoagulants, or concomitantly using antiplatelet drugs [18, 19]. The significance of the aforementioned BSSs (ATRIA, HAS-BLED, ORBIT) is in the assessment of individ-

Table 4. ORBIT and HAS-BLED scoring systems**
Tabela 4. *ORBIT i HAS-BLED sistemi bodovanja***

	Risk predictors *Prediktori rizika*	Scoring system *Sistem bodovanja*	Risk stratification *Stratifikacija rizika*
ORBIT *ORBIT*	Older age (≥ 74 years)/*Stariji uzrast (≥ 74 godine života)* Reduced haemoglobin/anemia *Snižen hemoglobin/anemija* Bleeding history *Anamnestički podatak o krvarenju* Insufficient kidney function *Bubrežna slabost* Treatment with antiplatelet drugs *Antitrombocitna terapija*	1 point for each risk factor *1 bod za svaki faktor rizika*	Low risk 0-2 *Nizak rizik 0-2* Intermediate risk 3 *Srednji rizik 3* High risk ≥ 4 *Visok rizik ≥ 4*
HAS-BLED *HAS-BLED*	Hypertension/*Hipertenzija* Abnormal renal and/or liver function/*Abnormalna bubrežna funkcija i/ili abnormalna hepatična funkcija* Stroke/*Šlog* Bleeding history* *Anamnestički podatak o krvarenju* Labile INR *Labilna vrednost INR* Elderly (≥ 65 years)/*Stariji uzrast (≥ 65 godina života)* Drugs or alcohol concomitant *Istovremena upotreba lekova, alkohola*	1 point for each risk factor *1 bod za svaki faktor rizika*	Low risk 0–1 *Nizak rizik 0–1* Intermediate risk 2 *Srednji rizik 2* High risk ≥ 3 *Visok rizik ≥ 3*

**(Modified from Wang C, Yu Ye, Zhu W, Yu J, Lip GY, Hong K. Comparing the ORBIT and HAS-BLED bleeding risk scores in anticoagulated atrial fibrillation patients: a systematic review and meta-analysis. Oncotarget [Internet]. 2017 Aug [cited 2022 Aug 9]; 8(65):[109703-109711]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5752553/)
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ual bleeding risk for patients beginning or continuing anticoagulant therapy [18]. The limitation of the aforementioned BSSs is that their prognostic performance is less than satisfactory [18, 19]. This is why contemporary guidelines of relevant medical organizations (ESC, AHA, ACC, HRS) do not yet encourage the use of currently available BSSs in deciding whether to administer antithrombotic therapy to a patient with AF [19]. BSSs for assessing bleeding risk in patients with AF on anticoagulant therapy should be used in order to recognize patients with a heightened bleeding risk who deserve more frequent reviews and monitoring during anticoagulant therapy [19, 24].

BSSs used for assessment of bleeding risk in patients with acute myocardial infarction treated with a percutaneous coronary intervention

Patients with acute myocardial infarction (AMI) treated with percutaneous coronary intervention (PCI) frequently experience bleeding events (from gastrointestinal to intracranial) which have a significant contribution to the morbidity and mortality of these patients [3, 28].

These bleeding events could be justified by the fact that these patients are usually older people who are prone to bleeding events, that they often have comorbidities that precipitate the occurrence of bleeding (unregulated hypertension, stress ulcers...), or that they use drugs that potentiate the occurrence of bleeding events (antiplatelet drugs, NSAID...) [3, 18].

Since the transradial (TR) approach in performing a PCI is associated with a lower incidence of bleeding events in comparison to the transfemoral approach, by favoring the TR approach, it is possible to reduce the rate of bleeding events in patients with AMI treated with PCI [3, 29].

Here we will mention CRUSADE, ACTION, and ACUITY-HORIZONS scoring systems which are used for the assessment of bleeding risk in patients with AMI treated with a PCI [3].

CRUSADE (an acronym for - "Can Rapid risk stratification of Unstable angina patients Suppress Adverse outcomes with Early implementation of the ACC/AHA Guidelines") is a BSS that includes 8 variables: gender, previous vascular disease history, personal history of diabetes, presence of heart failure symptoms, pulse, blood pressure, basic hematocrit, and creatinine clearance **(Table 5)** [3, 30, 31]. According to the results of Abu-Assi et al., CRUSADE is superior to ACTION and ACUITY-HORIZONS in predicting the occurrence of in-hospital bleeding in patients with ST segment elevation AMI (STEMI) and acute coronary syndrome (ACS) without ST segment elevation [3, 32].

Table 5. CRUSADE, ACUITY-HORIZONS and ACTION scoring systems
Tabela 5. *CRUSADE i ACUITY-HORIZONS sistemi bodovanja*

	CRUSADE (2009) *CRUSADE (2009)*	ACUITY-HORIZONS (2010) *ACUITY-HORIZONS (2010)*	ACTION-GWTG (2011) *ACTION-GWTG (2011)*
Study population *Studijska populacija*	CRUSADE quality improvement initiative/*CRUSADE inicijativa za poboljšanje kvaliteta* Derivation cohort (n=71.277) *Derivaciona kohorta (n=71,277)* Validation cohort (n=17.857) *Validaciona kohorta (n=17,857)*	ACUITY trial (n=13.819) *ACUITY trajal (n=13,819)* HORIZONS-AMI trial (n=3602) *HORIZONS-AMI trajal (n=3602)*	ACTION-GWTG registry *ACTION-GWTG registar* Derivation cohort (n=72.313) *Derivaciona kohorta (n=72,313)* Validation cohort (n=17.960) *Validaciona kohorta (n=17,960)*
Type of ACS *Tip AKS*	NSTEMI *NSTEMI*	NSTEMI (ACUITY) and STEMI (HORIZONS) *NSTEMI (ACUITY) i STEMI (HORIZONS)*	STEMI and NSTEMI *STEMI i NSTEMI*
Risk model variables *Varijable modela rizika*	Female sex/*Ženski pol* Diabetes/*Šećerna bolest* CrCl/*CrCl* Vascular disease *Vaskularna bolest* Hematocrit < 36% *Hematokrit < 36%* Heart failure/*Srčana slabost* Heart rate/*Srčana frekvencija* Systolic BP ≤ 110 mmHg *Sistolni KP ≤ 110 mmHg* Systolic BP ≥ 180 mmHg *Sistolni KP ≥ 180 mmHg*	Female sex/*Ženski pol* Age/*Starost* Baseline creatinine *Bazični nivo kreatinina* White blood cell count *Broj leukocita* Anemia/*Anemija* Clinical presentation *Klinička slika* Antiplatelet drugs *Antitrombocitni lekovi*	Age/*Starost* Body weight/*Telesna težina* Female sex/*Ženski pol* Warfarin use *Upotreba varfarina* Diabetes/*Šećerna bolest* Vascular disease *Vaskularna bolest* Baseline hemoglobin *Bazični nivo hemoglobina* Baseline creatinine *Bazični nivo kreatinina* Heart failure/shock *Srčana slabost/šok* Heart rate/*Srčana frekvencija* Systolic BP/*Sistolni KP* ECG changes/*EKG promene*
C-statistic *C-statistika*	Derivation cohort 0.71 *Derivaciona kohorta 0.71* Validation cohort 0.70 *Validaciona kohorta 0.70*	0.74	Derivation cohort 0.73 *Derivaciona kohorta 0.73* Validation cohort 0.71 *Validaciona kohorta 0.71*

CRUSADE, Can Rapid Risk Stratification of Unstable Angina Pectoris Suppress Adverse Outcomes With Early Implementation of the ACC/AHA Guidelines; ACUITY, Acute Catheterization and Urgent Intervention Triage strategY; HORIZONS, Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction; ACTION, Acute Coronary Treatment and Outcomes Network Registry; CrCl, creatinine clearance.

¶(Modified from Flores-Ríos X, Couto-Mallón D, Rodríguez-Garrido J, Garcia-Guimaraes M, Gargallo-Fernández P, Piñón-Esteban P et al.. Comparison of the performance of the CRUSADE, ACUITY-HORIZONS, and ACTION bleeding risk scores in STEMI undergoing primary PCI: insights from a cohort od 1391 patients. Eur Heart J Acute Cardiovasc Care [Internet]. 2013 Mar [cited 2022 Aug 9]; 2(1):[19-26]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3760576/)

CRUSADE, Can Rapid Risk Stratification of Unstable Angina Pectoris Suppress Adverse Outcomes With Early Implementation of the ACC/AHA Guidelines; ACUITY, Acute Catheterization and Urgent Intervention Triage strategy; HORIZONS, Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction; ACTION, Acute Coronary Treatment and Outcomes Network Registry; CrCl, klirens kreatinina; KP, krvni pritisak.

¶(Modifikovano prema Flores-Ríos X, Couto-Mallón D, Rodríguez-Garrido J, Garcia-Guimaraes M, Gargallo-Fernández P, Piñón-Esteban P et al.. Comparison of the performance of the CRUSADE, ACUITY-HORIZONS, and ACTION bleeding risk scores in STEMI undergoing primary PCI: insights from a cohort of 1391 patients. Eur Heart J Acute Cardiovasc Care [Internet]. 2013 Mar [cited 2022 Aug 9]; 2(1):[19-26]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3760576/)

ACTION (an acronym for - "Acute Coronary Treatment and Intervention Outcomes Network") is a BSS that includes 12 variables categorized into 3 groups: clinical background variables (gender, age, body weight, peripheral artery disease history, personal history of diabetes, personal history of warfarin treatment), current presentation variables (systolic blood pressure - SBP, heart rate, symptoms of shock or heart failure, ECG findings), and laboratory variables (baseline creatinine, baseline Hgb) **(Table 5)** [3, 31, 33, 34].

CRUSADE and ACTION BSSs were developed for predicting in-hospital bleeding risk in patients with an AMI (3).

ACUITY-HORIZONS was developed for predicting the bleeding rate within 30 days of AMI, and it includes the following variables: age, gender, clinical presentation, personal history of antiplatelet therapy, anemia, white blood cell count, and baseline creatinine **(Table 5)** [3, 31].

According to the results of Chen et al., ACUITY-HORIZONS is more reliable in comparison to other BSSs (ACTION, CRUSADE, HAS-BLED) in predicting the risk of serious bleeding (adopted definition of Thrombolysis In Myocardial Infarction) within 30 days of AMI treated with TR-PCI, whereby ACUITY-HORIZONS score above 17, Killip III and IV classes upon admission, and hypotension upon admission (SBP < 90 mmHg), positively predicted the risk of bleeding within 30 days of AMI, and greater mortality risk [3].

ACTION and ACUITY-HORIZONS were used for the assessment of the bleeding risk in patients with STEMI as well as AMI without ST - segment elevation (NSTEMI) [3].

BSSs used for assessing bleeding disorders

Hemostasis results from complex interactions between vascular, platelet, and coagulation factors, which contribute to the spontaneous termination of bleeding [35]. If any of the components mentioned above is impaired, this may result in a bleeding disorder [35–37].

Bleeding disorders are a heterogeneous group of rare, both hereditary and acquired diseases that manifest clinically through spontaneous bleeding in various tissues and organs [36, 37].

BSSs used for assessing bleeding disorders (also known as BATs - Bleeding Assessment Tools) have been developed in order to determine the likelihood of a patient having a bleeding disorder before performing a set of expensive and complex laboratory tests [4, 5, 38].

BATs are developed in order to improve the accuracy of a bleeding disorder diagnosis, but also to provide a more precise description of bleeding symptoms, as well as to estimate the risk of future bleeding events [38].

BATs consist of a standardized questionnaire and a scoring system used for calculating and interpreting the final score [6]. BATs are based on standardised anamnestic data, which is used for scoring the severity (and sometimes frequency) of bleeding events, thus directing adequate further diagnostic investigations [4, 5, 38].

When designing and performing a BAT it is crucial that medical professionals avoid misinterpreting trivial bleeding (which causes no emotional distress, does not impair social life and activities, and does not require medical treatment) for the symptoms of a bleeding disorder [6].

Vicenza BAT was the first validated BAT developed for establishing the diagnosis of von Willebrand disease type 1 (VWD1), and it was based on the International Society on Thrombosis and Hemostasis (ISTH) criteria [4]. Each bleeding symptom of the Vicenza BAT questionnaire is scored in a range between 0-3 according to its severity [5]. The later BATs were based on the original Vicenza BAT, which was modified (includence of the frequency of bleeding symptoms, shortening of administration time, development of a Pediatric Bleeding Questionnaire - PBQ) in order to improve the ease of evaluation, as well as their precision [4, 5]. Vicenza BAT score above 5 in adult females, as well as Vicenza BAT score above 3 in adult males, is associated with VWD phenotype [5].

MCMDM-1 VWD, MCMDM-1 VWD BQ (an acronym for "The European Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD Bleeding Questionnaire"), and the *Condensed MCMDM-1 VWD* were the earliest modifications of the Vicenza BAT [4, 5]. The issue with Vicenza BAT, MCMDM-1 VWD, and MCMDM-1 VWD BQ was their complexity and, therefore prolonged administration time (approximately 40 minutes), whereas introducing the Condensed MCMDM-1 VWD made the administration time significantly shorter (5-10 minutes) [5]. Each bleeding symptom in MCMDM-1 VWD and MCMDM-1 VWD BQ is scored in a range between -1 and +4 (-1 point is awarded for the lack of bleeding in response to hemostatic challenges), while the scoring range of the Condensed MCMDM-1 VWD is between -3 and +3 [4, 5]. MCMDM-1 VWD BS above 3 was associated with VWD in adults regardless of their sex [5].

ISTH-BAT (an acronym for "International Society on Thrombosis and Hemostasis Bleeding Assessment Tool") was developed for determining mild bleeding disorders in both adult and pediatric populations, and it was based on both severity and frequency of bleeding symptoms [5, 6, 38]. ISTH-BAT was merged from the previous four BATs in order to improve overall precision, flexibility, and ease of use [5]. Bleeding symptoms such as excessive bleeding after teeth extraction, surgery, and postpartum bleeding (hemostatic challenges) were removed from ISTH-BAT, thus avoiding the „negative scoring" [5, 6]. Sex differences in the normal range of ISTH-BAT scores (0-5 for adult females vs. 0-3 for adult males) are a consequence of the inclusion of gender-specific bleeding symptoms such as postpartum bleeding and menorrhagia, whereas the normal range for the pediatric population is between 0-2 [5]. Abnormal results of ISTH-BAT are considered to be ISTH-BAT ≥ 6 for females, ISTH-BAT ≥ 4 for males, and ISTH-BAT ≥ 3 for the pediatric population [5]. By using ISTH-BAT it is possible to accurately distinguish individuals with an inherited platelet function disorder from healthy individuals [38]. Since a high ISTH-BAT score in a thrombocytopenic patient does not exclude the presence of an inherited disorder, in this case, it is necessary to carefully assess medical history as well as blood cell morphology [38, 39]. ISTH-BAT score above 6 in patients with a mucocutaneous bleeding diathesis, for whom coagulation disorder or VWD1 diagnoses have been excluded (by using preliminary tests), indicates a 99% chance of being affected by an inherited platelet function disorder [38]. However, ISTH-BAT has been proven to be less accurate in differentiating VWD1 from inherited platelet disorders [38].

More recently a simplified self-testing modification of ISTH-BAT (Self-BAT) was introduced for patient self-administration [4].

ISTH encourages the use of ISTH-BAT in describing symptoms of bleeding disorders as well as their diagnostics in future research [6].

Conclusion

The contemporary guidelines of relevant medical organizations that address medical issues associated with bleeding encourage the proper use of BSSs in routine clinical practice.

Although there is a substantial number of BSSs already in use, the limitations of the existing BSSs urge the development of newer and improved BSSs.

The development of new BSSs as well as the enhancement of existing BSSs is a task that requires a comprehensive, multidisciplinary approach, along with a critical analysis of the existing BSSs.

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D-DIMER – ORIGIN AND CLINICAL SIGNIFICANCE

D-DIMER – POREKLO I KLINIČKI ZNAČAJ

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Summary

Introduction. D-dimer is formed during plasmin-mediated proteolysis of cross-linked fibrin; hence it serves as a biomarker of activated coagulation and fibrinolysis. **Clinical significance.** Measurement of D-dimer is most commonly used to exclude venous thromboembolism, and in the diagnosis of disseminated intravascular coagulation. For the diagnosis of venous thromboembolism D-dimer is part of the validated algorithm, which includes an assessment of clinical pre-test probability to guide further investigation. Due to very high negative predictive values, average levels of D-dimer are sufficient for ruling out venous thromboembolism in patients with low-medium pre-test clinical probability. However, in patients with high pre-test probability, the measurement of D-dimer is of limited value. Similarly, normal values of D-dimer reliably exclude disseminated intravascular coagulation. On the other hand, elevated values of D-dimer have low specificity for this condition and should be evaluated in a validated scoring system developed for the diagnosis of disseminated intravascular coagulation. Recently, measurement of D-dimer has been increasingly applied to assess the risk of venous thrombosis recurrence in women and to decide on the duration of anticoagulant therapy after the first unprovoked venous thrombosis. Elevated D-dimer level is an essential characteristic of COVID-19 - associated coagulopathy. The degree of coagulopathy and D-dimer levels correlate with the clinical severity of the disease and higher mortality, most likely reflecting increased activation of the coagulation system in the microcirculation of various organs, primarily the lungs. **Conclusion.** D-dimer is one of the most often used hemostasis test, validated so far for diagnosis of venous thromboembolism and disseminated intravascular coagulation.

Ky words: Fibrin Fibrinogen Degradation Products; Biomarkers; Fibrinolysis; Blood Coagulation; Risk Factors; Treatment Outcome; Prognosis; COVID-19; Anticoagulants

Sažetak

Uvod. D-dimer se formira kao rezultat razgradnje poprečno umreženog fibrina pod dejstvom plazmina i predstavlja biomarker aktivirane koagulacije i fibrinolize. **Klinički značaj.** Merenje D-dimera najčešće se koristi za isključivanje venskog tromboembolizma, kao i za dijagnostiku diseminovane intravaskularne koagulacije. Pri dijagnostici venskog tromboembolizma, D-dimer se određuje u okviru validiranog algoritma. Prvi korak u ovom algoritmu je procena kliničke verovatnoće postojanja tromboze standardizovanim pristupom, a rezultat te procene određuje daljnji postupak ispitivanja. Kod pacijenata sa niskim do srednjim stepenom kliničke verovatnoće nalaz normalne koncentracije D-dimera je dovoljan da isključi akutni venski tromboembolizan. Međutim, kod pacijenata sa velikom verovatnoćom ne preporučuje se određivanje D-dimera, pošto rezultat nije u mogućnosti da pouzdano isključi prisustvo tromboze. Slično tome, normalne vrednosti D-dimera pouzdano isključuju diseminovanu intravaskularnu koagulaciju. S druge strane, povišene vrednosti D-dimera imaju nisku specifičnost za ovu bolest i treba ih razmatrati u validiranom sistemu bodovanja na osnovu koga se postavlja dijagnoza diseminovane intravaskularne koagulacije. U poslednje vreme sve više se primenjuje merenje D-dimera, za procenu rizika od recidiva venske tromboze kod žena, pri donošenju odluke o trajanju antikoagulantne terapije nakon prve neprovocirane venske tromboze. Pokazano je da je kod muškaraca ova procena manje značajna. Povišen nivo D-dimera je suštinska karakteristika koagulopatije povezane sa COVID-19. Stepen koagulopatije i nivo D-dimera u korelaciji su sa kliničkom težinom bolesti i većim mortalitetom, što najverovatnije odražava povećanu aktivaciju koagulacionog sistema u mikrocirkulaciji različitih organa, pre svega pluća. **Zaključak.** D-dimer je jedan od najčešće određivanih testova hemostaze i njegova primena je validirana za dijagnozu venskog tromboembolizma i diseminovane intravaskularne koagulacije.

Ključne reči: D-dimer; biomarker; fibrinoliza; koagulacija; faktori rizika; ishod lečenja; prognoza; COVID-19; antikoagulantna terapija

Introduction

Coagulation system activation results in the generation of thrombin and conversion of fibrinogen into fibrin monomers. Once formed under the influence of thrombin, fibrin monomers spontaneously bind to each other, producing insoluble fibrin fibrils. The network of fibrin fibrils represents a basic structure of a blood clot either at the site of blood vessel wall injury or at the site of thrombus formation. Activated FXIII strengthens the fibrin network by cross-linking fibrin fibrils. In the next phase, the fibrinolytic enzyme plasmin breaks down the fibrin network leading to the dissolution of a blood clot. As a result of fibrinolytic degradation, fragments of different lengths and structures are formed, and they are generally called fibrin degradation products. D-dimer is a soluble cross-linked fibrin degradation

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Abbreviations

COVID-19 – Coronavirus disease of 2019
FXIII – Coagulation factor thirteen
ELISA – Enzyme-linked immunoassay
DIC – Disseminated intravascular coagulation
SARS-CoV-2 – Severe acute respiratory syndrome Corona virus 2
NPV – Negative predictive value
FDA – The Food and Drug Administration
CAC – COVID-19 associated coagulopathy

product resistant to further plasmin-mediated proteolysis. Theoretically, elevated blood concentrations of D-dimer are reliable indicators of fibrin deposits (thrombus) in the vasculature. However, the correct interpretation of D-dimer results may be hampered by the influence of numerous clinical, pre-analytical and analytical variables on this test.

Origin of D-dimer and laboratory determination

The polymerization of fibrin monomers in the elongated structure of fibrin fibrils is made possible owing to the complex structure of the fibrinogen molecule. It consists of two copies of three different polypeptide chains (A alpha, B beta, and gamma) linked by disulfide bonds and connected in the central E domain and two end D domains [1]. During the binding of fibrin monomers, the E domain from one monomer binds to the D domain from another, allowing fibrin dimers to form [2]. As the process of polymerization progresses, fibrin protofibrils are formed, consisting of two strands of polymerized fibrin monomers [3] **(Graph 1)**. Fibrin protofibrils intertwine in a complex structure, but they are still relatively fragile because they consist of fibrin monomers connected by non-covalent bonds. In the subsequent step, thrombin converts FXIII into an active transglutaminase that connects lysine residues from one chain of fibrin monomers with glutamic acid residues in the other chain. In this way, covalent bonds are formed between alpha and gamma chains of adjacent molecules of fibrin monomers strengthening fibrin structure [4]. As mentioned, fibrin fragments of different lengths and molecular mass are formed during the breakdown of the fibrin network under the action of plasmin.. D-dimer is a fibrin degradation product that consists of two D domains (DD fragment) from two adjacent fibrin monomer molecules covalently linked to each other by their gamma chains [5] **(Graph 1)**.

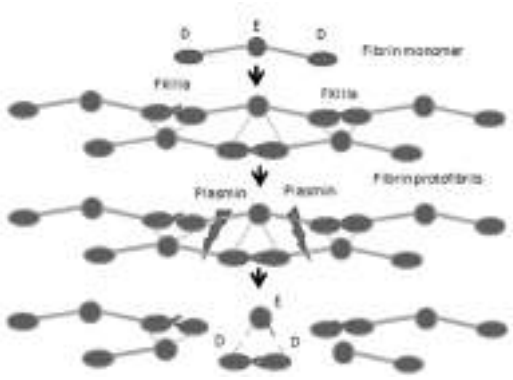
For the measurement of D-dimer concentration, specific monoclonal antibodies are used that recognize neoepitopes exposed on the D-dimer but not on the D domains of the intact fibrinogen molecule [6]. Therefore, the determination of D-dimer does not help recognize the existence of primary fibrinogenolysis or the breakdown of fibrin that is not cross-linked by the action of FXIIIa. It should be noted that monoclonal antibodies not only react with isolated DD fragments but also with this fragment contained in fibrin degradation products of higher molecular mass. It can be measured in whole blood by a rapid and semiquantitative method based on erythrocyte agglutination after adding a specific antibody. In plasma, D-dimer is mainly determined using the agglutination of latex particles coated with specific antibodies or the ELISA technique using the so-called "sandwich" principle. It has been shown that the ELISA method has the highest sensitivity in measuring the concentration of D-dimer. There are significant differences between commercial methods, especially of antibody characteristics, the threshold value for a positive result, and the characteristics of the control population in which the test was evaluated. Also, there is still no single international standard for D-dimer, which makes it practically impossible to compare the results obtained with different reagents [7].

Clinical significance of D-dimer measurement

Given that D-dimer is a product of the action of the fibrinolytic system on cross-linked fibrin, increased concentrations of this biomarker in the blood can be found in a range of diseases and conditions in which increased activation of the coagulation system occurs. However, in everyday clinical practice, measurement of blood D-dimer concentration is commonly used to exclude venous thromboembolism and in the diagnosis of disseminated intravascular coagulation (DIC). There are also convincing results indicating that D-dimer levels are predictive in assessing the risk of recurrent venous thrombosis in women but not in men. The significance of D-dimer measurement in most other indications is still unclear or unconfirmed. Elevated D-dimer levels are among the most significant characteristics of coagulopathy associated with COVID-19 and mainly correlate with the intensity of the inflammatory response to SARS-CoV-2 virus infection.

D-dimer in the diagnosis of venous thromboembolism

The concentration of D-dimer is increased in most people with acute venous thromboembolism, but elevated values are also found in healthy people in old age, in pregnancy, after trauma or surgical operations, in malignancies, chronic inflammatory diseases, and many other conditions. Therefore, elevated blood D-dimer values, determined by sensitive methods, have high sensitivity but low specificity for the presence of acute venous thromboembolism. On the other hand, average D-dimer values have a high negative predictive value (NPV) and exclude, with high probability, acute venous thromboembolism. It should be emphasized that the predictive value of D-dimer in ruling out thromboembolism largely depends on the laboratory method applied for D-dimer measurement. According to the specifications of the US Food and Drug Administration (FDA), methods for D-dimer measurement used for the diagnosis of venous thromboembolism should have a sensitivity of ≥95% and a negative predictive value of ≥97% [8]. Several studies have shown that D-dimer values of less than 500 ng/mL,



Graph 1. Formation and plasmin-mediated degradation of fibrin

Grafik 1. *Obrazovanje fibrina i njegova razgradnja pod dejstvom plazmina*

Thrombin converts fibrinogen molecules into fibrin monomers which spontaneously polymerize in a half-staggered format producing fibrin protofibrils. Activated FXIII additionally increases the tensile strength of fibrin fibers by cross-linking adjacent fibrin monomers. Plasmin causes lysis of fibrin fibers at multiple positions producing fibrin degradation products of different sizes. D-dimer consists of only two cross-linked D domains, and it is resistant to further proteolysis by plasmin.

Trombin prevodi fibrinogem u fibrin monomer koji spontano polimerizuje i na taj način formira fibrinske protofibrile. Aktivirani FXIII poprečno povezuje susedne fibrin monomere i time povećava čvrstinu krvnog ugruška. Plazmin lizira fibrinske niti na više mesta proizvodeći degradacione produkte fibrina različite veličine. D-dimer se sastoji od samo dva poprečno povezana D-domena i otporan je na daljnju razgradnju pod dejstvom plazmina.

if determined by a sensitive method, can exclude acute venous thromboembolism with a probability of about 97-99%. However, despite such a high NPV, the sole determination of D-dimer in the diagnosis of venous thromboembolism is not recommended, but this test should always be applied within the framework of a validated diagnostic algorithm that includes the assessment of the pre-test clinical probability of venous thromboembolism before determining the D-dimer level.

D-dimer in the diagnosis of disseminated intravascular coagulation

Disseminated intravascular coagulation may accompany various diseases and conditions, and is clinically characterized by the occurrence of thrombosis, bleeding or both thrombosis and bleeding at the same time, depending on the cause and extent of the coagulopathy. Given that the intravascular fibrin precipitation with widespread microvascular thrombosis is a cardinal characteristic of DIC, average D-dimer values determined by the reliable method practically exclude the presence of this type of coagulopathy [9]. On the other hand, similar to the situation with venous thromboembolism, elevated D-dimer values by themselves are not sufficient to confirm the diagnosis of DIC. D-dimer values are used as one of the diagnostic scoring system parameters that also include fibrinogen concentration, prothrombin time value and platelet count [10].

Determination of D-dimer to assess the duration of anticoagulant therapy

The optimal duration of anticoagulant therapy after the first episode of unprovoked venous thrombosis is not yet clearly defined. As many as 20-30% of patients experience recurrent venous thrombosis within five years after discontinuing anticoagulant therapy. In such persons, prolonged medicinal prophylaxis of venous thromboembolism would be fully justified. On the other hand, long-term administration of anticoagulant drugs in persons with a low risk of thrombosis recurrence carries an unnecessary risk of serious hemorrhagic complications. It has been shown that the concentration of D-dimer determined one month after the discontinuation of oral anticoagulant drugs, combined with data on the patient's gender, can be of great help in stratifying patients according to the estimated risk of recurrence of venous thromboembolism. In this way, the measurement of D-dimer provides an individualized approach to defining a long-term treatment strategy for patients with unprovoked venous thrombosis. It has been shown that the risk of repeated thrombosis during the first 12 months after discontinuation of anticoagulant therapy for women with elevated D-dimer values was 10%, and for those with average D-dimer values, about 5% [11]. This means that in women with high D-dimer values obtained one month after the initial period of anticoagulant therapy, re-introduction and long-term use of antithrombotic prophylaxis should be considered, while it can definitely be stopped in those with average D-dimer values. On the other hand, determination of D-dimer in the men showed limited value in the assessment of thrombosis recurrence risk.

D-dimer in Covid-19 coagulopathy

The coagulopathy that occurs in patients with COVID-19 ("COVID-19 associated coagulopathy-CAC"), is a thromboinflammatory condition that is characterized by the simultaneous presence of markers of inflammation and elevated concentrations of blood hypercoagulability markers with increased risk of thrombosis [12]. Although this coagulopathy in some of its laboratory characteristics resembles DIC seen in sepsis or other severe infections, CAC represents a separate entity with different pathogenesis and specific clinical manifestations. Unlike DIC, in which the clinical picture is dominated by a tendency to bleed, in coagulopathy associated with COVID-19, bleeding is rare. At the same time, the frequency of thromboembolic complications is high, especially in patients in intensive care units. In a systematic literature review and meta-analysis of the frequency of thromboembolism, Nopp et. al. reported the presence of thrombosis in 22.7% of critically ill persons treated in intensive care units and 7.9% of all hospitalized patients with COVID-19 [13]. Huang et al. reported that in patients who required admis-

sion to the ICU, D-dimer values (median 2.4 mg/L) were significantly higher than in patients with a milder form of the disease (median 0.5 mg/L, p=0.0042) [14]. Other authors also confirmed that the degree of coagulopathy correlates with the clinical severity of the disease and higher mortality, which is most likely a reflection of the above mentioned activation of the coagulation system in the microcirculation of various organs, primarily the lungs [15].

Conclusion

D-dimer is one of the most often used hemostasis test, validated so far for diagnosis of venous thromboembolism and disseminated intravascular coagulation.

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VON WILLEBRAND DISEASE – DETECTION, DIAGNOSTICS AND TREATMENT

VON VILEBRANDOVA BOLEST – PREPOZNAVANJE, DIJAGNOSTIKA I LEČENJE

Nebojša RAJIĆ

Summary

vonWillebrand disease is the most common inherited bleeding disorder, with mucocutaneous bleeding and menorrhagia as leading clinical manifestations. Cause of the bleeding diathesis is deficit or dysfunction of the vonWillebrand factor, plasma protein with important roles in adhesion of platelets to the site of vascular injury and transport and protection of coagulation factor VIII. Prevalance of the disease, according to different registries, is between 1 vonWillebrand disease on 100 persons to 1 vonWillebrand disease on 10000 persons. These data shows that detection of the disorder is not easy and that many cases are undiagnosed. That why bleeding assessment tools are developed and they are widely used for many years. Confirmation of the diagnosis is through laboratory testing. Some of tests are not easily accessible. Therapy of the disorder depends on goals of the treatment (stopping bleeding, prophylaxis or preoperative management). Last year, new guideline for diagnostics and treatment of vonWillebrand disease was published by experts of World Federation of Haemophilia, International Society of Thrombosis and Haemostasis, American Society of Hematology and National Hemophilia Foundation. In this paper, new recommendations for detection, diagnostics and treatment of the vonWillebrand disease, are presented.

Key words: von Willebrand Diseases; Diagnosis; Epidemiology; Genetics; Therapeutics

Sažetak

Von Vilebrandova bolest je najčešća nasledna bolest hemostaze koja se uglavnom manifestuje mukokutanim krvarenjima i menoragijama. U osnovi bolesti postoji deficit ili disfunkcionalnost Von Vilebrandovog faktora, proteina plazme, koji učestvuje u adheziji trombocita na mestu vaskularne lezije i kao nosač i zaštitinik faktora VIII koagulacije od autolize. Prevalencija bolesti iznosi, prema različitim registrima, od jedne osobe sa Von Vilebrandovom bolešću na 100 osoba u opštoj populaciji, do jedne osobe na 10.000 osoba. Ovi podaci ukazuju da detekcija bolesti nije laka i da postoje brojni nedijagnostikovani slučajevi. Zbog toga su i razvijeni *bleeding assessment tools,* koji su već godinama u upotrebi. Potvrda dijagnoze se vrši specifičnim laboratorijskim testovima, koji ponekad, nisu široko dostupni. Terapija Von Vilebrandove bolesti zavisi od tipa bolesti i cilja lečenja (zaustavljanje krvarenja, profilaksa ili priprema za hirurške intervencije). Prethodne godine objavljen je novi vodič za dijagnostiku i lečenje Von Vilebrandove bolesti, sastavljen od eksperata koje su predložile Svetska federacija za hemofiliju, Internacionalno udrženje za trombozu i hemostazu, Američko udruženje hematologa i američka Nacionalno fondacija za hemofiliju. U radu su predstavljene nove preporuke za detekciju, dijagnostiku i lečenje Von Vilebrandove bolesti.

Ključne reči: fon Vilebrandova bolest; dijagnoza; epidemiologija; genetika; terapija

Pathophysiology of VWD and genetics of VWF

VWD is an autosomal inherited bleeding disorder caused by the deficit or dysfunction of the von Willebrand factor (VWF). VWF is the plasma protein with great importance for platelet adhesion on the site of the primary vascular injury that also acts as carrier and protector of the coagulation factor VIII (FVIII). Bleeding in VWD could be due to impaired platelet adhesion or decreased plasma FVIII concentration. VWF is the protein consisting of multiple subunits in linear alignment, with variable length, so-called – multimers, sometimes over 20 million Daltons heavy and over two microns long [1]. After the synthesis in Weibel-Palade bodies, in the endothelium of the blood vessels, and in the megakaryocytes, the vWF molecule goes through complex intracellular processing; dimerization in the endoplasmatic reticulum, glycosylation in the endoplasmatic reticulum and Golgi complex, multimerization in the Golgi complex and storage in the Weibel-Palade bodies or alpha granules) [1]. During storage, VWF propeptide (VWFpp) is cut off from the molecule whose measurement, in the specific laboratory test, could have diagnostic value. VWF and FVIII in plasma circulate as loosely coiled protein complexes that do not interact strongly with platelets or endothelial cells under basal conditions [1]. However, at the site of the vascular injury, the VWF becomes tethered to the exposed subendothelium [1]. The conformation of the VWF multimers is changing, causing platelet adherence, activation, and aggregation on an activated platelet phospholipid surface [1]. Plasma VWF is primarily derived from the endothelium. The half-life of the VWF is around 12 h. VWF circulates as very large multimers that are subjected to physiological degradation by the metalloprotease ADAMTS13 [a Disintegrin-like and Metalloprotease domain (reprolysin type) with thrombospondin type 1 motif,

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Abbreviations

vWD	– von Willebrand disease
BAT	– bleeding assessment tools
ASH	– American Society of Hematology
ISTH	– The International Society of Thrombosis and He mostasis, The National Hemophilia Foundation, The World Federation of Hemophilia,
FVIII	– factor VIII
GPIb	– glycoprotein Ib (platelet)
VWF	– von Willebrand factor
vWFpp	– VWF propeptide
ADAMTS13	– [A Disintegrin-like And Metalloprotease domain (reprolysin type) with thrombospondin type 1 motif, member 13]
BT	– bleeding time; FVIII,
LD-RIPA	– low-dose ristocetin-induced platelet aggregation (concentration of ristocetin ≤0.6mg/mL);
N	– normal
PFA-100-CT	– platelet function analyzer closure time
PLT-VWD	– platelet-type VWD
RIPA	– ristocetin-induced platelet aggregation
VWF:Ag	– von Willebrand factor antigen
VWF:RCo	– von Willebrand factor ristocetin cofactor activity
DDAVP	– Desmopressin
I.U.	– International units

member 13). The plasma concentration of the VWF is increased by age, acute stress, inflammation, surgical interventions, and pregnancy. Hypothyroidism decreases the level of VWF. Afro-Americans and Africans have statistically higher levels of VWF, whereas persons with blood group type 0 have statistically lower levels of VWF [1].

The VWF gene is located on the 12th chromosome, close to the tip of the short arm -12p13.3 and it includes 178 kB and 52 exons [1]. Primates have a so-called "pseudogene" for VWF [1]. That is the sequence of 25 kB, which is located on the long arm of the 21st chromosome (21q11.2). It is analogous to the gen segment, which codes synthesis A1, A2, and A3 domains, which contain binding sites for platelet GpIb, collagen, and ADAMTS 13 [1]. This "pseudogene" is the "reservoir" for mutations, which could be transferred in the VWF locus on the 12th chromosome [1]. Types of genetic lesions are similar to the other genetic disorders. Multiple mutations, from large deletions to missense mutations affecting a single amino acid. There is no connection between a specific type of disease and genetic lesions. The original ISTH classification from 1994 connected specific genetic lesions with disease type, but it was abandoned in 2006 [1]. Genetic lesions in VWD are numerous and very complex, and sometimes the lesion is characteristic of a single family. The significance of detecting genetic mutations is diagnosis confirmation in problematic cases [2].

Epidemiology: WFH estimates that more than one in 1000 men and women has a bleeding disorder [3], but only 4% of that assumed number has a firmly set diagnosis. It isn't easy to estimate the actual prevalence of VWD, but it probably ranges from 1 in 100 to 1 in 10,000 [2–4]. So, it could be stated that VWD is the most common bleeding disorder with a prevalence of about 1%. Up to 3.5 million Americans may be affected. According to the United States Centers for Disease Control and Prevention (CDC), between 2012 and 2016, more than 14,600 men, women, and children had been seen at Hemophilia Treatment Centers (HTCs) for the treatment of VWD [4]. These statistics indicate that VWD is commonly unrecognized and that most people with VWD go undiagnosed [4]. The Republic of Serbia, with a population of around 6,8 million in 2021, should have around 68000 people with VWD. This number is far larger than the number from the Serbian Registry of patients with bleeding disorders (author's opinion).

Classification: The ISTH classification of VWD from 1994 and revised in 2006 is shown in **Table 1.**

Diagnosis: Detection and diagnosis of VWD require detailed and systematic personal and family history directed towards discovering the symptoms of bleeding or data of the existence of a known bleeding disorder. VWD usually presents with signs of mild or moderate mucocutaneous bleeding, less frequently with symptoms of gastrointestinal or genitourinary tract bleeding, except menorrhagia, which is very common (more than 60% with type

Table 1. Classification of von Willebrand disease (1)
Tabela 1. *Klasifikacija von Willebrandove bolesti (1)*

Type *Tip*	Description *Opis*
1	Partial quantitative deficiency of VWF/*Delimičan količinski nedostatak VWF*
2	Qualitative VWF defect/*Kvalitativni poremećaj VWF*
2A	Decreased VWF-dependent platelet adhesion with selective deficiency of high-molecular-weight multimers *Smanjena adhezija trombocita u zavisnosti od VWF sa selektivnim nedostatkom velikih multimera*
2B	Increased affinity for platelet GPIb/*Povećan afinitet trombocita za GPIb*
2M	Decreased VWF-dependent platelet adhesion without selective deficiency of high-molecular-weight multimers *Smanjena adhezija trombocita usled VWF bez selektivnog nedostatka velikih multimera*
2N	Markedly decreased binding affinity for FVIII/*Izrazito smanjen afinitet za FVIII*
3	Virtually complete deficiency of VWF/*Praktično potpun nedostatak VWF*

FVIII - factor VIII; GPIb- glycoprotein Ib (platelet); VWF - von Willebrand factor

Table 2. Laboratory results in different VWD subtypes (1)*
Tabela 2. *Laboratorijske analize za različite podvrste VWD*

	Normal *Normalno*	Type 1 *Tip 1*	Type 2A *Tip 2A*	Type 2B *Tip 2B*	Type 2M *Tip 2M*	Type 2N *Tip 2N*	Type 3 *Tip 3*	PLT-VWD
VWD:Ag	N	L, ↓ or/*ili* ↓↓	↓ or/*ili* L	↓ or/*ili* L	↓ or/*ili* L	N or/*ili* L	Absent *Odsutan*	↓ or/*ili* L
VWD:RCo	N	L, ↓ or/*ili* ↓↓	↓↓ or/*ili* ↓↓↓	↓↓	↓↓	N or/*ili* L	Absent *Odsutan*	↓↓
FVIII	N	N or/*ili* ↓	N or/*ili* ↓	N or/*ili* ↓	N or/*ili* ↓	↓↓	1-9 IU/dL	N or/*ili* L
RIPA	N	Often N *Često N*	↓	Often N *Često N*	↓	N	Absent *Odsutan*	Often N *Često N*
LD-RIPA	Absent *Odsutan*	Absent *Odsutan*	Absent *Odsutan*	↑↑↑	Absent *Odsutan*	Absent *Odsutan*	Absent *Odsutan*	↑↑↑
PFA-100 CT	N	N or/*ili* ↑	↑	↑	↑	N	↑↑↑	↑
BT	N	N or/*ili* ↑	↑	↑	↑	N	↑↑↑	↑
PLT	N	N	N	↓ or/*ili* N	N	N	N	↓
vWF multimer pattern/*Sadržaj vWF multimera*	N	N	Abnormal *Abnormalan*	Abnormal *Abnormalan*	N	N	Absent *Odsutan*	Abnormal *Abnormalan*

*Expected laboratory values in VWD. The symbols and values represent prototypical cases. In practice, laboratory studies in certain patients may deviate slightly from these expectations. L, 30–50 IU/dL; ↓, ↓↓, ↓↓↓, relative decrease; ↑, ↑↑, ↑↑↑, relative increase; BT - bleeding time; FVIII, factor VIII activity; GPIb, platelet glycoprotein Ib complex; LD-RIPA, low-dose ristocetin-induced platelet aggregation (concentration of ristocetin ≤0.6 mg/mL); N, normal; PFA-100-CT, platelet function analyzer closure time; PLT-VWD, platelet-type VWD; RIPA, ristocetin-induced platelet aggregation; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity.

Očekivane laboratorijske vrednosti u VWD. Simboli i vrednosti predstavljaju standardne slučajeve. U praksi, laboratorijske studije kod određenih pacijenata mogu neznatno odstupiti od ovih vrednosti. L, 30–50 IU/dL; ↓, ↓↓, ↓↓↓, relativno smanjenje; ↑, ↑↑, ↑↑↑, relativno povećanje; BT - vreme krvarenja; FVIII, aktivnost faktora VIII; GPIb, trombocitni glikoprotein Ib kompleks; LD-RIPA, agregacija trombocita izazvana malim dozama ristocetina (koncentracija ristocetina ≤0,6 mg/mL); N, normalno; PFA-100-CT, analizator funkcije vremena zgrušavanja trombocita; PLT-VWD, VWD tipa trombocita; RIPA, ristocetinom indukovana agregacija trombocita; VVF, von Vilebrandov faktor; VWF:Ag, antigen von Vilebrandovog faktora; VWF:RCo, aktivnost von Vilebrandovog faktora- ristocetin kofaktor

3 VWD) [5]. Hemarthrosis is almost exclusively noticed in type 3 VWD but relatively frequent; around 40% of patients with type 3 had bleeding in joints [5]. Detailed physical examination implies searching for signs of bleeding (ecchymosis, petechiae) and other signs like lymphadenopathy, hepatosplenomegaly, or jaundice. In the further diagnostic algorithm, it is necessary to perform a so-called screening laboratory test of hemostasis: bleeding time, activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), and fibrinogen. These tests aim to exclude other disorders of hemostasis. The next step is to perform the confirmatory tests-measuring VWF antigen (VWF: Ag), VWF: RCoF (VWF Activity), and FVIII level. For definitive diagnosis confirmation, especially for type 2, sometimes performing additional tests is necessary, like the detection of VWF multimers (electrophoresis in gel), RIPA test, a test of binding for collagen (VWF: CB), and genetic test **(Table 2).**

Aiming to improve the detection of VWD, which is underdiagnosed worldwide, many questionnaires (bleeding scores) were developed. These bleeding assessment tools (BAT) search for persons who should be sent for further investigation in specialized centers. According to new recommendations proposed by ASH, ISTH, WFH, and NHF, BAT - ISTH score should be used extensively in the primary healthcare system (general practice) [6]. BAT - ISTH was validated because it increases the rate of disease detection. However, it is time-consuming, and some populations are left "under the radar" (children and adult men) [7]. Also, new recommendations suggest the usage of the so-called self-BAT (filled out by the patient online). Although they usually give slightly overestimated results, self-BAT could be very useful [8].

Laboratory diagnostic in VWD is rather complex and many tests are not easily available and affordable. Standard tests are:

– Measurement VWF: Ag is an immunoassay (ELISA or LIA) that measures the concentration of VWF protein in plasma. Test results should be reported in international units (IU), either as international units per deciliter (IU/dL) or as international units per milliliter (IU/mL). Most laboratories choose IU/dL, because it is similar to the conventional manner of reporting clotting factor assays as a percentage of normal. [1]

– VWF:RCoF (VWF:Activity) could be performed by 5 different tests (turbidimetric, automated or not, slope of the curve during platelet aggregation with ristocetin, ELISA or test with monoclonal antibodies). Results also in IU/dL. All those tests have wide intra- and interlaboratory variability. Value of the VWF:RCo can vary up to 10 IU/dL.

– FVII assay – one stage or chromogenic

– Factors like stress during specimen collection, inadequate anticoagulants, anemia or polycythemia, inadequate thawing of the frozen specimens can influence results.

Other test for diagnosis confirmation and differentiation between certain types of the disease, are:

– VWF multimer analysis: VWF multimer analysis is a qualitative assay that depicts the variable concentrations of different-sized VWF multimers by using sodium dodecyl sulphate–protein electrophoresis followed by detection of the VWF multimers in the gel, using a radiolabelled polyclonal antibody or a combination of monoclonal antibodies [1]

– Ristocetin-induced platelet aggregation (RIPA) test: Low-dose RIPA is carried out in platelet-rich plasma, using a low concentration of ristocetin (usually < 0.6 mg/mL, although ristocetin lots vary, resulting in the use of slightly different ristocetin concentrations). This low concentration of ristocetin does not cause VWF binding and aggregation of platelets in samples from normal persons, but it does cause VWF binding and aggregation of platelets in samples from patients with type 2B VWD [1]

– VWF:CB – measures the capacity for binding to collagen

– platelet function analyzer (PFA) 100 closure time,

– genetic tests and others.

Besides results from different tests, it is very important to calculate the ratio:

VWF:RCo/VWF:Ag < 0.5-0.7 = dysfunctional VWF = type 2 (A,B ili M).

New recommendation - (ASH, ISTH, NHF i WFH) suggests newer assays that measure platelet-binding activity of VWF (eg, VWF:GPIbM, VWF:GPIbR) over the VWF ristocetin cofactor assay (VWF:RCo) (automated or non automated assay) for the diagnosis of VWD (conditional recommendation based on low certainty in the evidence [6].

Ratio vWF:RCoF/vWFAg - served for many years, as the main tool in rapid differentiation between typel and type 2 vWD. Newer tests, like measuring platelet-binding activity of vWF, are expensive and unaffordable for many laboratories. It is questionable how much this recommendation will be implemented in real-world practice.

New recommendation - VWF level of < 0.30 IU/mL regardless of bleeding, and for patients with abnormal bleeding, a VWF level of < 0.50 IU/mL to confirm the diagnosis of type 1 VWD (strong recommendation based on low certainty in the evidence [6].

It was common practice to label patients with vWF levels 0.3-0.5 IU/ml as “low level vWF" not “vonWillebrand disease”, because some of them never had severe bleeding and at one occasion that lower levels were recorded. It was rather common for patients with blood group O and females during or after menstrual bleeding. But new recommendations [6] suggest that all of them should be labeled as von-Willebrand disease. Makris et Hermans asked about the elevation of VWF, which is expected with aging. Will it mean that the diagnosis of these persons should be changed during life? [7]. This issue is still to be solved.

Treatment

There are three treatment strategies:

1. Elevation of plasma concentration of VWF by releasing endogenous VWF from endothelial cells – desmopressin
2. Application of pharmaceutical agents – a concentrate of VWF and FVIII
3. Application of pharmaceutical agents that promote hemostasis without changing VWF concentration (antifibrinolytics, topical agents).

Desmopressin

Desmopressin (DDAVP) is a synthetic derivative of the anti-diuretic hormone vasopressin. Desmopressin stimulates the release of VWF from endothelial cells through its agonist effect on vasopressin V2 receptors [1]. The mechanism by which desmopressin increases plasma concentration of VWF is probably through cyclic adenosine monophosphate (cAMP)-mediated release of VWF from endothelial cell Weibel-Palade bodies [1]. Standard dosing of desmopressin is 0.3 μg/kg i.v. in 30–50 mL of normal saline over 30 min, with peak increments of FVIII and VWF 30–90 min after the infusion. Desmopressin could cause severe allergic reactions, hypertension, hypotension, and hyponatremia [1]. Some persons don’t react to desmopressin, some type 2 and all type 3 patients. Subsequent doses have lesser effect. So, desmopressin is ideal for minor bleeding and minor surgical interventions.

New recommendations propose against the use of desmopressin before surgery, without previous tests of efficacy [6].

Concentrates of VWF

In the Republic of Serbia, Haemate P® and Wilate®, plasma-derived concentrates, are available.

Haemate-P®, a lyophilized concentrate of purified VWF and FVIII, contains other plasma proteins, including fibrinogen and albumin. In Haemate-P®, the quantity of the large, most hemostatically active multimers of VWF is decreased compared to fresh plasma. When reconstituted at the recommended volume, each milliliter of the product contains 50–100 IU/mL VWF: RCo and 20–40 IU/mL FVIII activity.

Ratio FVIII/VWFR: Co in Haemate P® is 2.7:1 and 1:1 in Wilate®.

Dosing depends on the content of VWF in the product and the target level of VWF in plasma.

There are two types of treatment with concentrates:

1. On-demand treatment is applied after a bleeding episode and before surgical interventions when the target VWF level is 50-100 IU/dL. The duration of treatment in major surgical interventions is from 7 to 14 days, for minor interventions is from 1 to 5 days

2. Prophylaxis with VWF concentrates is possible for persons with severe and frequent bleeding (usually type 3). There is no strict regimen, and it is individually tailored. Rather small proportion of vWD patients are on prophylaxis [1].

New recommendation-prophylaxis should be more frequent especially for type 3.

Pharmaceutical agents to promote hemostasis

Antifibrinolytic drugs aminocaproic acid (EACA) and tranexamic acid are agents that inhibit the conversion of plasminogen to plasmin, inhibiting fibrinolysis and thereby helping to stabilize clots that have formed [1]. The dosing of EACA is four to five grams orally or intravenous; after that, one gram/hour or four to six grams every six hours.

Tranexamic acid is dosed with 10 mg/kg body mass every eight hours (0.5 grams or 1.0 grams every eight hours oral or intravenous). The excretion of both agents is through the kidneys.

Bovine thrombin and fibrin glue are topical agents in use.

Conclusion

VonWillebrand disease is still fascinating, complex and a disease with many unsolved issues concerning detection, diagnosis and treatment. Although published guidelines are improving, we could expect further changes in the years to come.

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PRIMARY IMMUNE THROMBOCYTOPENIA
PRIMARNA IMUNOLOŠKA TROMBOCITOPENIJA

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PRIMARY IMMUNE THROMBOCYTOPENIA IN ADULTS – DISEASE CONSIDERATIONS

KARAKTERISTIKE IMUNSKE TROMBOCITOPENIJE KOD ODRASLIH

Nada SUVAJDŽIĆ VUKOVIĆ[1,2], Nikola PANTIĆ[1], Zlatko PRAVDIĆ[1], Mirjana CVETKOVIĆ[1], Nikica SABLJIĆ[1] and Mirjana MITROVIĆ[1,2]

Summary

Introduction. Primary immune thrombocytopenia is a chronic acquired autoimmune disorder that is characterized by isolated thrombocytopenia (<100 x10^9/L) and the absence of any underlying cause. **Treatment of primary immune thrombocytopenia.** While splenectomy has a curable potential, it carries long-term risk of infection and thromboembolic complications. Therefore, the use of splenectomy has declined with the advent of rituximab and agonists of thrombopoietin receptors. The efficacy of rituximab is good for the short-term outcome, and the majority of patients will relapse. On the other hand, agonists of thrombopoietin receptors induce remission in only 10-30% of patients after treatment discontinuation, and long-term treatment is often required. **Health - related quality of life**. Immune thrombocytopenia and its treatments may affect the entire spectrum of patients' lives, encompassing daily activities, emotional health, energy level, fatigue, and work productivity. Primary immune thrombocytopenia World Impact Survey was conducted to discern how immune thrombocytopenia and associated treatments affect patient lives. Concerns about unstable platelet count, low energy levels, inability to exercise, and reduced participation in hobbies and work had the greatest negative impact. While most patients reported "good health", nonetheless half of patients reported a negative impact on their emotional well-being that worsened with increasing burden of disease and was often substantial. **Conclusion.** Although several important improvements have been made in immune thrombocytopenia treatment algorithms, there is still room for improvement. One of the possible options could be early, intensive treatment of immune thrombocytopenia, which might reduce the risk of disease progression and consequently improve patients' quality of life.

Key words: Thrombocytopenia; Adult; Therapeutics; Splenectomy; Rituximab; Quality of Life; Treatment Outcome

Sažetak

Uvod. Primarna imunska trombocitopenija je hronično, autoimunsko oboljenje koje se karakteriše izolovanom trombocitopenijom (< 100 x 10^9/L) u odsustvu drugih uzročnih faktora. **Lečenje primarne imunološke trombocitopenije.** Splenektomija je kurativna terapijska opcija. Međutim, povišen rizik od infekcija i tromboembolijskih komplikacija nakon operacije su doživotni. Sa otpočinjanjem upotrebe rituksimaba i agonista trombopoetinskih receptora za terapiju imunske trombocitopenije, učestalost splenektomije je opala. Rituksimab je efikasna terapijska opcija, ali je odgovor nakon njegove primene kratkotrajan i kod većine pacijenate se javi relaps. Sa druge strane pacijenti na agonistima trombopoetinskih receptora postižu remisiju i stabilan odgovor nakon obustave terapije u samo 10−30%, pa je kod većine neophodna doživotna terapija. **Kvalitet života povezan sa zdravljem.** Imunska trombocitopenija i način na koji je lečimo može uticati na različite aspekte pacijentovog života kao što su svakodnevne aktivnosti, emocionalno stanje, nivo energije, osećaj zamora i produktivnost na poslu. *I-WISh* anketa je sprovedena sa ciljem da ispita prave razmere ovog problema. Pokazano je da na kvalitet života najviše utiču briga zbog nestabilnog broja trombocita, nemogućnost da treniraju, osećaj smanjene energije, nemogućnost aktivnog učestvovanja u hobijima i na poslu. Naime, najveći broj pacijenata je naveo da je „dobrog zdravlja", ali se čak polovina njih požalila na negativan uticaj bolesti na emotivno blagostanje. **Zaključak.** Iako je terapija imunske trombocitopenije značajno unapređena poslednjih godina, prostora za dodatno poboljšanje i dalje ima. Jedna od mogućih opcija svakako je rano intenzivnije lečenje ITP-a kojim bi se sprečilo napredovanje bolesti i posledično poboljšao pacijentov kvalitet života.

Ključne reči: trombocitopenija; odrasli; terapija; splenektomija; rituksimab; kvalitet života; ishod lečenja

Introduction

Primary immune thrombocytopenia (ITP) in adults is a chronic acquired autoimmune disorder characterized by increased platelet destruction and impaired platelet production. Both processes are driven by an aberrant immune response to platelets and megakaryocytes, particularly at the level of T cells. It is characterized by isolated thrombocytopenia < 100 x10^9/L and the absence of any underlying cause [1]. Its incidence in adults has been estimated to be 1-4 per 100 000 persons. The mean age of adults at diagnosis

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Abbreviations

ITP – primary immune thrombocytopenia
HRQoL – Health-related quality of life
TPO-RAs – agonists of thrombopoietin receptors
PC – Platelet counts
TFR – Treatment-free response
TGF-beta – Transforming growth factor beta
I-WISh – ITP World Impact Survey

in Europe is 50 years, and the incidence of ITP increases with age [2]. Many patients with ITP exhibit no symptoms or minimal bruising, but some experience serious bleeding. These signs and symptoms of the disease and treatment side effects might affect the patient's health-related quality of life (HRQoL) [3–5]. Additional factors such as age, lifestyle, and comorbidities affect the bleeding risk and should be taken into account when treatment decisions are made.

The curable potential of ITP treatments

Some of the ITP treatments have curable potential. Splenectomy is an effective therapy for steroid - refractory or steroid - dependent ITP, which induce long-lasting remission in 60-70% of patients [6]. However, there are no reliable predictors of splenectomy response [7], and long-term risks of infection and thromboembolic complications must be considered [8–10]. With the advent of rituximab and agonists of thrombopoietin receptors (TPO-RAs), the use of splenectomy has declined. Moreover, the new guidelines recommend waiting 12-24 months before splenectomy to allow for spontaneous or therapy-induced remissions [11]. The efficacy of rituximab is good for the short-term outcome. Most patients will relapse, and the percentage of long-term responders is about 20%. Rituximab combined with three dexamethasone cycles provided similar responses to splenectomy. Moreover, the duration of ITP < 2 years, achieving a complete response, and being female were associated with better long-term remission [12]. TPO-RAs, both eltrombopag and romiplostim, induce platelet production by stimulating megakaryocytopoiesis. Eltrombopag and romiplostim are shown to increase platelet counts (PC) and prevent major bleeding in 80-90% patients with ITP. They also improve patients' quality of life and they are well tolerated. Thrombocytopenia typically recurs upon discontinuation of both of them [1, 13]. Notably, randomised, controlled trials comparing TPO-RAs have not been performed yet.

Treatment-free response induced by TPO-RAs

Several studies have shown that TPO-RAs induce remission and stable response in 10-30% of patients after treatment tapering and discontinuation [14]. However, the majority of these studies did not find any clinical or treatment characteristics that may predict the treatment-free response (TFR). This TFR may be interpreted as a recovery of immunological tolerance via several mechanisms: increased or improved T- and B-regulatory cell activity increased TGF-beta, which mediates the increased T- and B-regulatory cell activity, and reversal of Fc receptor balance towards inhibitory FcRIIb [15, 16]. It is hypothesized that TPO-RAs exhibit tolerogenic activities due to increased platelet and microparticle mass (exposure to high-dose antigen with immunosuppressive effects) [17].

When and how to taper patients off TPO-RAs

Several practical recommendations are generated on when and how to taper patients off TPO-RAs. Patients suitable for tapering are those with stable PC > 50 x10^9/L for at least 6 to 12 months without concomitant therapy for ITP. Conversely, tapering is not advised in patients requiring high-dose TPO-RA and PC < 50 x 10^9/L; in ITP that was previously hard to manage; treatment duration with TPO-RAs less than 6-12 months; with a high risk of bleeding if treatment stopped; on concurrent antiplatelets or anticoagulants required and significant comorbidities and risk of recurrent infections. TPO-RAs should be re-initiated in case of clinical bleeding and PC < 30 x10^9/L at ≥ 1 assessment [14, 18].

The role of early, intensive treatment of ITP

Several recent studies showed that intensive medical treatment administered early in the disease course might improve or even cure ITP [19, 20]. Moreover, based on Newland's study, romplostim received supplementary approval in the USA for adults with ITP who have failed first-line therapy, without any specific restriction on time from diagnosis. Precisely, Newland' s study was an open-label, single-arm phase 2 trial of 75 adults with ITP diagnosed ≤ 6 months prior who had an insufficient response to first-line treatment, including corticosteroids. The median time from ITP diagnosis to study enrollment was 2.2 months. On the primary endpoint, the median number of months with platelet response (≥ 50 x 10^9/L) was 11 months during the 12-month treatment period (95% CI: 10, 11), with a median time to first platelet response of 2.1 weeks (95% CI: 1.1, 3.0). Additionally, 93% of patients achieved one or more platelet responses during the 12-month treatment period. On the secondary endpoint, 32% of patients achieved remission for at least 6 months, defined by maintaining a PC ≥ 50 x 10^9/L in the absence of romiplostim and any concomitant/rescue therapy for ITP. The biological rationale for this early and intensive treatment is that it rapidly abrogates the priming immune response during the initial disease before the memory response has been established. The memory response is more difficult to treat [21].

Health-related quality of life

ITP requires lifelong treatment and monitoring in a substantial proportion of adult patients, thereby negatively impacting the patients' HRQoL [3, 22]. Improvement in HRQoL parameters has been identified as an essential treatment objective in the updated ITP guidelines [11, 23]. However, physicians often underestimate or ignore HRQoL parameters,

as the principal treatment goal for ITP is to treat or prevent bleeding [3, 24].

The impact of ITP and its treatments on patients' HRQoL may affect the entire spectrum of patients' lives, encompassing daily activities, emotional health, energy level, fatigue, and work productivity. Compared with age-matched and sex-matched controls, patients with ITP have lower work productivity, more physician visits, and are more likely to take sick leave [3, 5, 24]. Interestingly, with the current standard of care, HRQoL appears to deteriorate in the first year after diagnosis and improve later in the disease course [5]. This suggests that patients become so accustomed to a life with reduced HRQoL that they may think it is their "normal".

Recently, the ITP World Impact Survey (I-WISh) was conducted to discern how ITP and associated treatments affect patient lives and to evaluate how aligned patient and physician perceptions are with regard to symptoms, HRQoL, and disease management [5]. The I-WISh survey, which included ten questions HRQoL questionnaire, is a novel, pervasive tool which identifies the degree of impact that ITP has on patients' HRQoL, emotional health, social, personal and works life. Concerns about unstable PC, low energy levels, inability to exercise, and reduced participation in hobbies and work had the most significant negative impact. While most patients reported "good health", half of them reported a negative impact on their emotional well-being that worsened with increasing disease burden and was often substantial [5]. I-WISh also identified concerns about bruising in 35% of patients with ITP to the extent of wearing long-sleeve clothing. As initially reported, ITP has an impact on sex life as well as on social life in 60% and 70% of patients, respectively. ITP negatively impacted work and productivity; almost half of the patients reported that they had reduced, or seriously considered reducing, their working hours due to ITP. Up to 25% declined a promotion, had to take early retirement, or quit their job [5]. Health professionals working with patients with ITP need to consider the impact of physical appearance and fatigue on HRQoL and adjust their management accordingly.

Differences between physician and patient viewpoints on HRQoL have been reported. However, I-WISh survey reflected the increased awareness of reduced HRQoL and low energy in patients with ITP by physicians who were experienced in the care of ITP [5]. On the other hand, data from India showed that fatigue was reported as severe by 60% of patients at diagnosis, and only 33% of physicians perceived it as a severe problem [24]. Even more, 58% of physicians felt their patients experienced anxiety about PC, whereas this was identified as an issue in 53% of patients. Similar proportions of physicians and patients identified fear of dying from ITP as an issue (48% vs. 41%, respectively) [3, 5, 24].

Conclusion

Discontinuation of agonists of thrombopoietin receptors is achievable in 25–30% of selected responding patients after tapering, but the reliable predictors for treatment-free response are not yet available. Treatment-free response could be attributed to the recovery of immunological tolerance. The advantages of discontinuation are reduced costs and toxicity and improved patients' health-related quality of life. Besides, several recent studies showed that early, intensive treatment of primary immune thrombocytopenia might reduce the risk of disease progression. However, more extensive studies with a longer follow-up are needed to assess the efficacy and safety of earlier use of agonists of thrombopoietin receptors and to better select patients for tapering. Improvement of health-related quality of life generally requires a multidimensional approach and should be tailored for the individual patient. A shared-decision model could also help in ensuring that the treatment goals of patients and physicians are entirely aligned.

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SUPPORTIVE THERAPY
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TUMOR LYSIS SYNDROME

SINDROM LIZE TUMORA

Ivanka PERČIĆ

Summary

Introduction. Tumor lysis syndrome is an emergency condition requiring prompt recognition and treatment. It's a consequence of spontaneous or therapy-induced cellular death leading to the release of intracellular ions and metabolic products of purine bases into the bloodstream. **Pathophysiology.** The characteristic metabolic derangement comprises hyperkalemia, hyperphosphatemia, hyperuricemia, and hypocalcemia. These metabolic changes can lead to kidney failure, arrhythmia, and seizures. **Epidemiology, classification and risk assessment.** The incidence of tumor lysis syndrome varies between different types of tumors, but it is most common in hematologic malignancies. According to Cairo – Bishop Classification, tumor lysis syndrome can be defined as laboratory tumor lysis syndrome and clinical tumor lysis syndrome. **Preventive measures and treatment of tumor lysis syndrome.** Frequent laboratory monitoring is obligatory in patients with intermediate and high risk of tumor lysis syndrome. Preventive measures are based on vigorous hydration and administration of medication to control serum uric acid levels. When clinical tumor lysis syndrome develops, additional treatment, including renal replacement therapy, is needed for the correction of metabolic disturbances. **Conclusion.** Tumor lysis syndrome is a potentially fatal complication in patients with suffering from malignancies. Early recognition of patients at risk and administration of prophylactic and therapeutic measures improves outcomes for these patients.

Key words: Tumor Lysis Syndrome; Signs and Symptoms; Classification; Epidemiology; Pathology; Therapeutics; Risk Assessment

Sažetak

Uvod. Sindrom lize tumora je urgentno stanje koje zahteva rano prepoznavanje i lečenje. Nastaje kao posledica spontane ili terapijom uzrokovane ćelijske smrti što dovodi do oslobađanja intracelularnih jona i metaboličkih produkata purinskih baza u krvotok. **Patofiziologija.** Karakteristični metabolički poremećaj u sindromu lize tumora obuhvata hiperkalijemiju, hiperfosfatemiju, povišene serumske vrednosti mokraćne kiseline i hipokalcemiju. Ove promene mogu prouzrokovati akutnu bubrežnu insuficijenciju, aritmije i neurološke smetnje. **Epidemiologija, klasifikacija i procena rizika.** Učestalost sindroma lize tumora zavisi od vrste maligne bolesti, ali je najčešća kod hematoloških maligniteta. Po važećoj *Cairo–Bishop* klasifikaciji se razlikuju laboratorijski i klinički sindrom lize tumora. **Prevencija i lečenje sindroma lize tumora.** Kod bolesnika sa intermedijarnim i visokim rizikom je neophodno često laboratorijsko i kliničko praćenje. Osnova prevencije je obilna hidracija i primena lekova koji smanjuju serumski nivo mokraćne kiseline. U slučaju razvoja kliničkog sindroma lize tumora, uz preventivne mere je potrebno lečenje metaboličkih poremećaja uključujući zamenu bubrežne funkcije. **Zaključak.** Sindrom lize tumora je potencijalno smrtonosna komplikacija kod bolesnika sa malignim bolestima. Primena preventivnih i terapijskih mera poboljšava prognozu za ove bolesnike.

Ključne reči: sindrom lize tumora; znaci i simptomi; klasifikacija; epidemiologija; patologija; terapija; procena rizika

Introduction

Tumor lysis syndrome (TLS) is a hematologic - oncologic emergency requiring immediate recognition and treatment. It is the consequence of spontaneous or therapy-related rapid cellular death leading to the release of a waste amount of intracellular ions and metabolic products of purine bases into the bloodstream [1, 2]. The incidence of TLS varies between different types of tumors and depends on patient and tumor characteristics. Arrhythmias and acute kidney failure are the two most common life-threatening complications resulting from these metabolic changes. Hyperuricemia and hyperphosphatemia cause kidney failure, hyperkalemia, and hypocalcemia cause arrhythmia. The mortality can be as high as 79% in patients with acute myeloid leukemia in induction treatment [3]. Treatment modalities such as corticosteroids, conventional chemotherapy, and targeted agents can be causative agents [4–6].

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Abbreviations
TLS – Tumor lysis syndrome
ATP – Adenosine triphosphate
LTLS – Laboratory tumor lysis syndrome
CTLS – Clinical tumor lysis syndrome
LDH – Lactate dehydrogenase
G6PD – Glucose-6-phosphate-dehydrogenase
RRT – Renal replacement therapy

Pathophysiology

Rapid spontaneous or, more frequently, therapy-related cell death occuring in the first few days after treatment leads to the release of intracellular contents of malignant cells into the bloodstream [2]. The characteristic metabolic derangement in TLS comprises hyperkalemia, hyperphosphatemia, hyperuricemia, and usually secondary hypocalcemia.

Hyperkalemia is the earliest laboratory alteration occurring after 12-24 hours [7]. It is the result of the death of cancerous cells. However, another mechanism contributes to elevated potassium levels. Potassium level in cells is regulated by a sodium/potassium channel requiring adenosine triphosphate (ATP) for its function. Since cell metabolism is increased after staring chemotherapy, it leads to the expenditure of ATP, the amount of ATP left for the regulation of potassium gradient is decreased, and potassium leaves the cancer cell even before lysis occurs [7, 8]. Acute kidney failure contributes to hyperkalemia which, alongside hypocalcemia, leads to arrhythmia.

The level of phosphate is up to four times higher in malignant cells in respect to normal cells [9]. Phosphate released in the course of cancer cell death leads to hyperphosphatemia. Phosphate binds calcium leading to the formation of calcium-phosphate. If the calcium-phosphate product exceeds $60 mg^2/dl^2$, the risk of calcium-phosphate precipitation in the tubules increases [10, 11]. When compensatory mechanisms, like decreased tubular absorption, are overwhelmed, deposition of calcium-phosphate and uric acid crystals leads to acute kidney failure [11]. Hypocalcemia secondary to hyperphosphatemia can lead to cardiac dysfunction and tetany.

Hyperuricemia is caused by the catabolism of purine bases into hypoxanthine and xanthine, and then to uric acid via xanthine oxidase. Uric acid is poorly soluble in water, leading to crystal formation and deposition in distal tubules, especially in acidic urine [2]. On the other hand, uric acid causes endothelial dysfunction, inflammation, and oxidative stress contributing to kidney damage [2, 9]. Some mammals, but not humans, can metabolize uric acid into allantoin via urate oxidase. Allantoin is more soluble in water and therefore easily excreted in the urine. Hyperuricemia is usually detected after 48 to 72 hours [7].

Epidemiology, classification and risk assessment

To estimate and report the incidence of TLS, uniform definition and diagnostic criteria were needed. The first definition and classification were given by Hande and Garrow [1]. In their study on the incidence and types of TLS on 102 patients with "intermediate" and "high-grade" lymphoma, Hande and Garrow defined TLS as laboratory TLS (LTLS) and clinical TLS (CTLS) [1]. LTLS comprised laboratory alterations characteristic of TLS but without metabolic disturbances and the need for a specific treatment, whereas CTLS is characterized by potentially life-threatening complications requiring prompt treatment [1]. In this group of patients, the incidence of LTLS was 42%, whereas CTLS was diagnosed in 6% of patients [1].

Cairo and Bishop modified the Hande – Garrow definition [2]. Patients with two or more laboratory abnormalities reported on the same day, occurring three days prior and up to seven days after the initiation of cancer treatment, are diagnosed with LTLS [2]. Patients with LTLS and one or more clinical criteria are diagnosed with CTLS. The Cairo – Bishop definitions of LTLS and CTLS are shown in **Table 1** and **Table 2**.

The incidence of TLS varies between different types of tumors. In a retrospective analysis of 951 cancer patients, the overall incidence of LTLS was 9.3%, and CTLS 6.7% [12]. TLS was most common in hematologic patients, most notably patients with acute leukemia and multiple myeloma. Two-thirds of patients with LTLS developed CTLS. Aggressive non-Hodgkin's lymphoma, like diffuse large B-cell lymphoma and Burkitt lymphoma, acute myeloid, and lymphoblastic leukemia, are most commonly associated with TLS [10]. TLS is less common in solid tumors except for highly chemosensitive tumors like bulky small cell lung cancer and metastatic germ cell carcinoma [4].

Table 1. Cairo – Bishop Definition of laboratory tumor lysis syndrome (2)
Tabela 1. *Cairo–Bishop definicija laboratorijskog sindroma lize tumora (2)*

Criteria for laboratory tumor lysis syndrome diagnosis *Kriterijumi za postavljanje dijagnoze laboratorijskog sindroma lize tumora*	
Uric acid *Mokraćna kiselina*	≥ 476 μmol/l or 25% increase from baseline/≥ 476 μmol/l or 25% increase in respect to initial values
Phosphorus/*Fosfor*	≥ 2.1 mmol/l (children), ≥ 1.45 mmol/l (adults) or 25% increase from baseline/≥ 2.1 mmol/l (children), ≥ 1.45 mmol/l (adults) or 25% increase in respect to initial values
Potassium/*Kalijum*	≥ 6.0 mmol/l or 25% increase from baseline/≥ 6.0 mmol/l or 25% increase in respect to initial values
Calcium/*Kalcijum*	≤ 1.75 mmol/l or 25% decrease from baseline/≤ 1.75 mmol/l or 25% reduction in respect to initial values

Table 2 Cairo – Bishop Definition of clinical tumor lysis syndrome (2)
Tabela 2. *Cairo–Bishop definicija kliničkog sindroma lize tumora (2)*

Criteria for clinical tumor lysis syndrome diagnosis *Kriterijumi za postavljanje dijagnoze kliničkog sindroma lize tumora*
Creatinine ≥ 1.5 above ULN/*Kreatinin ≥ 1.5 iznad gornje granice referentnih vrednosti*
Cardiac involvement (arrhythmia, sudden death)/*Zahvatanje kardiovaskularnog sistema (aritmije, iznenadna smrt)*
Neurologic involvement (seizure, tetany)/*Zahvatanje nervnog sistema (napadi, tetanija)*

ULN – upper limit of normal

Clinical manifestations of TLS reflect the underlying metabolic derangements. Nausea, vomiting, diarrhea, decreased level of consciousness, arrhythmia, seizures, tetany, and finally, death may occur [13].

The development of TLS can lead to life-threatening complications. Therefore, early risk assessment, prevention, and treatment are essential. The risk of TLS is dependent on disease, patient and treatment characteristics. Besides type and chemosensitivity, other tumor characteristics predisposing to TLS are large tumor burden and high proliferative rate [14]. Cairo et al. proposed a three-phase risk assessment system [15]. The first step is the assessment of the presence of LTLS. Secondly, tumors are graded as low, intermediate, and high risk based on age, stage, presence of bulky disease, white blood count, and lactate dehydrogenase (LDH) level [15]. The third step is the presence of renal failure. Combining these three components, patients are defined as low, intermediate, or high risk for TLS development [15]. Volume depletion, older age, male gender, preexisting renal failure, and elevated uric acid levels are recognized as patient-related risk factors [15, 16]. Treatment with certain medications influences the risk of TLS. Besides conventional cycle-specific drugs, novel targeted agents like venetoclax, bortezomib, rituximab, and even treatment with corticosteroids, may induce TLS [4-6].

Preventive measures and treatment of TLS

Although potentially fatal, TLS can be prevented. The first step is to identify patients at risk, correct electrolyte disturbances and volume depletion prior to chemotherapy. Frequent laboratory monitoring is mandatory in patients at intermediate and high risk of TLS, including serum uric acid, potassium, phosphorus, calcium, urea, creatinine, and LDH levels every 6-12 hours, depending on the risk [4, 11, 14]. Fluid intake and urine output should be closely monitored. Calcium, potassium, and nephrotoxic drug intake should be limited if not discontinued. Preventive measures are based on vigorous hydration and the administration of medication to control serum uric acid levels.

Hydration is a cornerstone in the prevention of TLS and should be initiated prior to cytotoxic treatment. The recommended volume is not less than 3000mL/day for adults aiming to maintain an adequate urine output of at least 100 ml/hour to increase intravascular volume, renal perfusion, and decrease uric acid and calcium –phosphate deposition in the tubules [4, 7, 11]. Diuretics to maintain urine output can be used cautiously when volume repletion is completed. Patients with underlying cardiac disease require precise monitoring and the initiation of loop diuretics, if fluid overload is suspected [9].

Urine alkalization decreases uric acid crystal formation and deposition in renal tubules. However, it promotes the deposition of calcium-phosphate crystals; therefore, it is no longer recommended [11].

Allopurinol, an inhibitor of xanthine-oxidase, should be administered a few days before, during chemotherapy, and 3-7 days after, until the normalization of uric acid level. By inhibiting xanthine-oxidase, the formation of uric acid from xanthine and hypoxanthine is decreased. However, it does not affect the uric acid already formed [17]. The dose varies between 100-400 mg/m^2 divided into three doses with a maximum dose of 800 mg/day [7, 9, 11]. The dose should be adjusted in case of renal failure and use of other medications [11].

Febuxostat is a xanthine-oxidase inhibitor routinely used for the treatment of gout. In FLORENCE, a randomized, double-blind study on 346 patients, researchers compared the efficacy of allopurinol in three different doses to a fixed dose of febuxostat. The study showed the superiority of fixed-dose febuxostat in achieving serum uric acid level control over allopurinol [18]. In a meta-analysis that included six studies, allopurinol and febuxostat had similar response rates and incidences of TLS [19]. The use of febuxostat may be considered in patients with chronic renal failure, since dose adjustment is not required [9].

Rasburicase is a recombinant form of the enzyme urate-oxidase that converts uric acid into more water-soluble allantoin [20]. A phase III multicenter study assessed patients at high risk of hyperuricemia and TLS receiving either rasburicase or rasburicase followed by allopurinol and allopurinol alone [21]. Plasma uric acid level control was significantly better with rasburicase than with allopurinol [21]. Normalization of uric acid serum level is achieved four hours after rasburicase treatment [21, 22]. Rasburicase can be administered as a single dose of 3 mg or 0.2 mg/kg daily for up to five days in high-risk adults [7]. Rasburicase does not require dose adjustment in renal failure, and allergic reactions are seldom compared to allopuri-

nol. However, rasburicase cannot be used in patients with glucose-6-phosphate-dehydrogenase (G6PD) deficiency; it is costly and therefore less available.

Clinical TLS is an emergency. Vital parameters should be continuously monitored. Uric acid, LDH, electrolytes, renal function, arterial blood gases, and electrocardiogram should be assessed every six to 24 hours [4]. All mentioned prophylactic measures should be continued. Additional treatment is needed for the correction of metabolic disturbances. Glucose and insulin, loop diuretics, oral binding resins, beta2agonists, and renal replacement therapy (RRT) treat hyperkalemia. Hyperphosphatemia can be treated with hydration, phosphate chelating agents, and RRT. The administration of calcium gluconate is indicated in case of hyperkalemia-induced cardiac toxicity and symptomatic hypocalcemia. Indications for RRT are worsening renal failure, persistent electrolyte disturbances despite medical treatment, and hypervolemia [11].

Conclusion

Tumor lysis syndrome is a preventable but potentially fatal emergency in hematology-oncology. Early recognition of patients at risk and administration of prophylactic and therapeutic measures improves outcomes for these patients.

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FEBRILE NEUTROPENIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES – DEFINITION, DIAGNOSIS AND MANAGEMENT

FEBRILNE NEUTROPENIJE KOD BOLESNIKA SA HEMATOLOŠKIM MALIGNITETIMA – DEFINICIJA, DIJAGNOZA I PRISTUP

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Summary
Intensive chemotherapy/radiotherapy in cancer, especially with hematologic malignancies, causes cellular injury and suppression of inflammatory responses, which increase the risks of neutropenia and febrile episodes. Absolute neutrophil count < 1×10^9/L is considered neutropenia, with absolute neutrophil count $< 0.5 \times 10^9$/L or $< 1 \times 10^9$/L that is expected to decrease to $< 0.5 \times 10^9$/L in the next 48 hours considered severe neutropenia, while absolute neutrophil count $< 0.1 \times 10^9$ is referred as profound neutropenia. Febrile episodes are usually defined as oral temperature > 38.3°C or two consecutive readings > 38.0°C lasting more than 1 hour. Although there is the possibility of non-infection–caused febrile neutropenia, most episodes are caused by infections. Febrile neutropenia is a clinical emergency that requires prompt management. Despite advances in therapy in recent years, febrile neutropenia remains a common complication in chemotherapy causing serious clinical results, including death. The administration of empirical antibacterial therapy has been successful in the management of febrile neutropenia since its launching 50 years ago. The wide application of broad-spectrum antibiotics has effectively decreased the mortality of febrile neutropenia patients. Neutropenic patients who remain febrile despite 4-7 days of broad-spectrum antibacterial therapy are at a high risk of invasive fungal infection. Empirical antifungal therapy with Amphotericin B or Caspofungin in persistently febrile neutropenic patients and other high-risk patients has shown to reduce the risk of invasive fungal infection by 50 – 80% and the risk of fungal infection-related mortality by 23 – 45%. Lipid formulations which improve the therapeutic ratio of the traditional formulation are available
Key words: Febrile Neutropenia; Hematologic Neoplasms; Diagnosis; Therapeutics; Empirical Research; Anti-Infective Agents; Antifungal Agents; Fever; Risk Factors

Sažetak
Intenzivna hemoterapija/radioterapija kod karcinoma, posebno kod hematoloških maligniteta, dovodi do oštećenja ćelija i supresije inflamatornog odgovora, što povećava rizik od neutropenije i febrilnih epizoda. Apsolutni broj neutrofila $< 1 \times 10^9$/L smatra se neutropenijom, sa apsolutnim brojem neutrofila $< 0,5 \times 10^9$/L ili $< 1 \times 10^9$/L za koje se očekuje da će se smanjiti na $< 0,5 \times 10^9$/L u narednih 48 sati smatra se teškom neutropenijom, dok apsolutni broj neutrofila $< 0,1 \times 10^9$ označen je kao duboka neutropenija. Febrilne epizode se obično definišu kao oralna temperatura > 38,3° C ili dva uzastopna očitavanja > 38,0° C koja traju više od jednog sata. Iako je moguće da febrilna neutropenija nije uzrokovana infekcijom, većina epizoda je uzrokovana infekcijama. Febrilna neutropenija je hitno kliničko stanje koje zahteva hitan tretman. Uprkos napretku u terapiji poslednjih godina, febrilna neutropenija ostaje česta komplikacija primenjene hemioterapije koja izaziva ozbiljne kliničke komplikacije, uključujući smrt. Upotreba empirijske antibiotske terapije bila je uspešna u lečenju febrilna neutropenija od njenog lansiranja pre 50 godina. Upotreba antibiotika širokog spektra efikasno je smanjila mortalitet pacijenata sa febrilnom neutropenijom. Upotreba empirijske antibiotske terapije bila je uspešna u lečenju febrilne neutropenije od njenog lansiranja pre 50 godina. Pacijenti sa neutropenijom koji ostaju febrilni uprkos antibakterijskoj terapiji širokog spektra u trajanju 4−7 dana pod visokim rizikom su od invazivne gljivične infekcije. Pokazalo se da empirijska antifungalna terapija amfotericinom B ili kaspofunginom kod pacijenata sa upornom febrilnom neutropenijom i drugih visokorizičnih pacijenata smanjuje rizik od invazivne gljivične infekcije za 50–80% i rizik od smrtnosti od gljivične infekcije za 23–45%. Dostupne su lipidne formulacije amfotericina B koje poboljšavaju terapijski uspeh, u poređenju sa konvencionalnim amfotericinom B, uz značajno smanjenje nefrotoksičnosti i hepatotoksičnosti.
Ključne reči: febrilna neutropenija; hematološke neoplazme; dijagnoza; terapija; empirijska istraživanja; antibiotici; antimikotici; groznica; faktori rizika

Introduction

Fever is the main sign of infection in neutropenic patients and frequently may be the only evidence of infection. The pattern of fever in neutropenia is non-specific and not pathognomonic of any type of infections or non-infectious process and can be suppressed by the antipyretic effects of drugs such as corticosteroids [1]. Neutropenia, resulting from cytotoxic chemotherapy is the most common risk factor for se-

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vere infections in hematological malignancies. The duration of neutropenia also contributes significantly to the risk of serious infections. This risk is significantly greater with lower neutrophil counts, such that 100% patients with ANC <100 cells/μl lasting 3 weeks or more develop documented infections **(Table 1)**.

Neutropenic patients who remain febrile despite 4-7 days of broad spectrum antibacterial therapy are at a high risk of invasive fungal infection [1, 2]. Empirical antifungal therapy with amphotericin B or caspofugin in persistently febrile neutropenic patients and other high risk patients has shown to reduce the risk of invasive fungal infection by 50-80% and the risk of fungal infection related mortality by 23-45% in 1980's. Lipid formulations which improve the therapeutic ratio of the traditional formulation are available [1–3].

Although fever is a frequent sign of infection, noninfectious causes must also be considered: pyrogenic drugs (cytosine arabinoside), blood products, allergic reactions and underlying malignancy are potential sources of fever.

Definition of fever and neutropenia

Neutropenia is defined as an absolute neutrophil count (polymorphonuclear cells plus band forms) of 500/ml or less. From a practical standpoint, patients with ANC between 500 and 1000 cells/ml, and rapidly falling because of recent chemotherapy, are also considered neutropenic [1].

Impaired host defenses in hematological malignancies

Patients with hematological malignancies are immunocompromised as a result of the underlying malignancy or due to the therapeutic interventions employed to manage it. Some malignancies are associated with specific immune defects that predispose to infections with particular pathogens [4]. Patients with acute leukemia have an increased risk of severe gram-negative bacterial infections as a result of quantitative or functional neutropenia. Patients with chronic lymphocytic leukemia and multiple myeloma are susceptible to invasive bacterial infections from staphylococci and streptococci, especially pneumococcus. Conversely patients with lymphoma have abnormalities of the cellular immune system resulting in an increased risk of viral infections (e.g. herpes simplex) and fungal infections (e.g. Cryptococcus). Therapeutic interventions such as corticosteroids, chemotherapy, stem cell transplant, and radiation also produce deficiencies in the host defense [2–4]. Neutropenia, resulting from cytotoxic chemotherapy is the most common risk factor for severe bacterial infections in hematological malignancies. Impaired T-cell function in patients undergoing allogeneic stem cell transplant is associated with an increased susceptibility to invasive viral infections. Other therapy induced alterations in host colonization such as disruption of natural skin and mucosal barriers and interference with nutrition also increase the risk of infection.

Some hematological procedures such as venepunctures, bone marrow aspiration and insertion of central venous access devices, disrupt the integument and provide a nidus for colonization [3,4]. The degree of neutropenia, either as a consequence of disease or therapy, is directly related to the incidence of serious bacterial and fungal infection. There is a significant increase in the incidence of serious infection once ANC falls below 500 cells/ml. Patients with ANC below 100 cells/ml are at the highest risk of infection.

The duration of neutropenia also contributes significantly to the risk of serious infections. This risk is significantly greater at lower neutrophil counts, such that 100% patients with ANC < 100 cells/ml lasting 3 weeks or more develop documented infections.

Qualitative defects in neutrophil function have been described in hematological malignancies. These include defects in chemotaxis, phagocytosis, bactericidal capacity, and absence of respiratory burst that accompanies phagocytosis. Additionally, chemotherapeutic agents including corticosteroids can decrease phagocytosis and neutrophil migration [5–7].

Spectrum of microbial pathogens in hematological malignancies

Over the last three decades, there has been a significant change in the spectrum of infections in neutropenic patients with acute leukemia. In the early 1950s and 1960s staphylococcus aureus was the most frequent isolate in immunosuppressed patients. With the introduction of beta-lactamase-resistant antistaphylococcal pencillins, gram-negative bacilli became the predominant bacterial organisms including Escherichia coli, Klebsiella species and Pseudomonas aeroginosa. Since the 1980s, several studies have collectively demonstrated a shift in the etiology of bacterial infections from a predominance of gram-negative pathogens to gram-positive cocci [6–8]. Factors responsible for this shift include the widespread use of indwelling central venous access devices, use of intensive chemotherapy toxic to the upper and lower gastrointestinal mucosa, use of quinolone-based antibacterial chemoprophylaxis that suppress aerobic gram-negative bacilli colonizing the gastrointestinal tract but fail to suppress the microaerophilic gram-positive cocci and the use of histamine H2 receptor blockers, which reduce gastric pH and promote overgrowth with oropharyngeal gram-positive microflora.

Clinically important gram-positive pathogens include the viridans group streptococci such as S. mitis and S. mileri, Enterococcus species such as the glycopeptide resistant strain of E. faecium and the coagulase negative staphylococci that comprise the predominant normal skin microflora. Staphylococcus epidermidis is the species most often isolated from patients with coagulase negative staphylococcal bacteremia. The Enterococcal species, E. faecalis and E. faecium have emerged as virulent pathogens due to the acquisition of antibiotic resistant plasmids. Vancomycin-resistant and aminoglycoside resistant strains are being found increasingly in outbreaks among seriously ill patients. Anaerobes play a lesser role in primary infections in neutropenic fever, but

Table 1 Parameters for defining the degree of risk for the development of FN (adapted from NCCN guideline 2019)
Tabela 1. *Parametri za procenu rizika za razvoj FN (preuzeto sa NCCN smernica 2019)*

LOW RISK/*NIZAK RIZIK* (probability of FN < 20%/*verovatnoća FN < 20%)*
Outpatient status of the patient at the time of onset of high fever/*Visoka febrilnost nastala u vanbolničkim uslovima*
Absence of acute comorbidities/*Odsustvo akutnih komorbiditeta*
Expected short duration of severe neutropenia (<7 days)/*Očekivana kratkotrajna teška neutropenija (< 7 dana)*
Good performance status (ECOG 01)/*Dobar performans status (ECOG 01)*
Absence of liver insufficiency/*Odsustvo jetrene insuficijencije*
Absence of renal insufficiency/*Odsustvo bubrežne insuficijencije*
MASCC score³ 21/*MASCC skor³ 21*
HIGH RISK/*VISOK RIZIK* (probability of FN > 20% */verovatnoća FN > 20%)*
Expected prolonged severe neutropenia (ANC<1.0 x 10^9/L for more than 7 days) *Očekivano produženo trajanje teške neutropenije (ABN < 1.0 x 10^9/L duže od 7 dana)*
Significant clinical comorbidity, or clinically unstable patient *Značajni klinički komorbiditeti ili klinički nestabilan pacijent*
Failure to achieve complete remission in patients with acute leukemia *Nepostizanje kompletne remisije kod pacijenata sa akutnom leukemijom*
Presence of pneumonia or other complex infections at the time of hematological diagnosis *Prisustvo upale pluća ili neke druge teške infekcije u momentu postavljanja hematološke dijagnoze*
Grade 3-4 mucositis*/Gradus 3-4 mukozitisa*
Hospital status of the patient at the time of high fever/*Razvoj visoke febrilnosti u bolničkim uslovima*
Alemtuzumab therapy (CampathR)/*Almetuzumab terapija (CampathR)*
Liver failure (aminotransferase values increased 5 times) *Jetrena insuficijencija (5 puta povišene vrednosti aminotransferaza)*
Renal insufficiency (creatinine clearance < 30 mL/min)/*Bubrežna insuficijencija (klirens kreatinina < 0,30 ml/min)*
MASCC score < 21/*MASCC skor < 21*

are responsible for mixed infections in the mouth and perianal area. Clostridia perfringens, C. septicum, and C. tertium have been associated with serious infections. Infection with Bacillus species has been associated in patients with indwelling silastic catheters. Fungi are major pathogens, especially in patients with prolonged neutropenia and who receive protracted courses of antibiotics. The predominant fungal pathogens are Candida species, Aspergillus species, C. neoformans, and the Phycomycetes. Although less common, the mucoraceae (Mucor, Absida, and Rhizpus species) can cause pulmonary disease or rhinocerebral mucomycosis. Parasite or viral infections are important primary infections or cause secondary complications. Pneumocystic carinii is an important cause of pneumonia, especially in patients receiving corticosteroids. Herpes simplex virus (HSV), varicellazoster virus (VZV) and cytomegalovirus (CMV) are the most prevalent among viral pathogens. Other viruses that are benign in the normal host, such as adenovirus respiratory syncytial virus (RSV) and human herpes virus type 6 (HHV 6) can cause significant respiratory infections in the immunocompromized host. A special problem is infection with the SARS-CoV-2 virus in patients with hematological malignancies, whether it is an isolated COVID infection, or whether it is associated with a secondary bacterial or invasive fungal infection.

Initial evaluation of febrile neutropenic patients

There are two important considerations in the initial evaluation. Neutropenia markedly alters the host's inflammatory response, making it difficult to detect infection. Second, an undetected and untreated infection can be rapidly fatal in the neutropenic patient. The classic signs and symptoms of infections are often missing. Therefore a careful history and a detailed physical examination to look for subtle signs of inflammation are necessary. This examination must be frequently repeated in persistently febrile patient. It is very important to determine the degree of risk of patients for the development of febrile neutropenia **(Table 1)**. Even subtle evidence of inflammation must be considered as sign of infection. Minimal perianal erythema and tenderness may rapidly progress to perianal cellulitis. Minimal erythema or serous discharge at the site of a Hickman catheter may herald tunnel or exit site infection. Particular attention should be paid to sites that are frequently infected or serve as foci for dissemination of infection such as oropharynx, lung, paranasal sinuses, perineum, and vascular

Table 2. Empirical antibiotic therapy for febrile neutropenia
Tabela 2. *Empirijska antibiotska terapija za febrilnu neutropeniju*

	Monotherapy/*Monoterapija*
	Imipenem/cilastatin Meropenem Piperacillin/Tazobactam Cefepime Ceftazidime*
	Combined therapy/*Kombinovana terapija*
Intravenous *Intravenska*	Aminoglycoside (amikacin or gentamicin) + antipsudomonal penicillin with or without a β lactamase inhibitor (piperacillin/tazobactam) *Amikoglikozidi (amikacin ili gentamicin) + antipseudomonasni penicilini sa ili bez inhibitora β laktamaze (piperacillin/tazobactam)*
	Aminoglycoside + broad – spectrum cephalosporin (cefepime or ceftazidime) *Amikoglikozidi + cefalosporini širokog spektra (cefepim ili ceftazidim)*
	Ciprofloxacin + antipseuomonas penicillin (piperacillin/tazobactam) *Ciprofloksacin + antipseudomonasni peniclin (piperacillin/tazobactam)*
	Vancomycin is added as needed to both mono and combined therapy, in selected patients *Vakomicin se dodaje po potrebi i kod mono i kod kombinovane terapije kod određenih pacijenata*
	Antibiotic therapy low risk patients ***Antibiotska terapija kod pacijenata sa niskom rizikom***
Oral *Oralna*	Ciprofloxacin + amoxicillin/clavulanic acid (Panaclav®) In the case of penicillin allergy use clindamycin *Ciprofloksacin+ amoksicili/ klavulanska kiselina (Panaklav®)* *U slučaju alergije na peniclinske preparate koristiti klindamicin*

catheter insertion sites. Prior to initiating empirical antibiotic therapy, at least two sets of blood culture and cultures from other appropriate sites (e.g. throat, urine, stool) should be obtained for bacteria and fungal organisms. In patients with central venous catheters, simultaneous cultures should be obtained from the catheters as well as from a peripheral site. Cultures should be repeated daily while patients remain febrile. All febrile neutropenic patients should undergo chest radiography to identify pulmonary lesions. Radiographs or CT scans of paranasal sinuses should be performed in patients in whom these sites are potential sources of infection. Imaging techniques such as CT, MRI, ultrasonography and radionuclide imaging and invasive procedures such as bronchoscopic examination, lung, liver or skin biopsy may be extremely useful in identifying sites of infection [2–6]. However, the presence of thrombocytopenia often precludes the use of invasive diagnostic techniques.

Management of febrile neutropenia

The prompt initiation of empirical antibiotics in febrile neutropenia has been the most important advance in the management of the immunocompromised host. Since the widespread use of empirical antibiotics, the overall survival rate for febrile neutropenic patients is more than 90%. The first effective treatment for febrile neutropenia was demonstrated in the landmark trial by Schimpff and consisted of a combination of carbenicillin and gentamycin [6]. Treated patients with P. aeruginosa infection had dramatically improved survival compared to historic controls. Some investigators have argued that combination therapy broadens the spectrum of activity, retards the development of resistance and offers the potential of synergistic activity particularly against gram-negative bacilli. Since the 1980s, the development of broad-spectrum antipseudomonal antibiotics with high serum bactericidal level to minimal inhibitory concentration ratio has led to reevaluation of the need for combination antibiotic therapy. The practice of combination antibiotic therapy was changed by the introduction of newer highly active third generation cephalosporins such as ceftazidime which had a broad spectrum of anti-gram-negative activity including activity against P. aeruginosa. Further, the addition of an aminoglycoside did not consistently improve the clinical outcome in neutropenic patients. Other agents such as imipenem/cilastin, meropenem and cefepime have been studied as empirical monotherapy in febrile neutropenia [4–6]. The major concern about monotherapy has been on the betalactam resistance among coagulase-negative staphylococci, viridans group streptococci, enteric gram-negative bacilli and methicillin resistant S. aureus (MRSA). Fourth generation cephalosporins such as Cefepime are active against most penicillin and ceftazidime resistant viridans group streptococci and against gram-negative bacilli that produce group 1 betalactamases including enterobacter and proteus **(Table 2)**. The overall response rates for cefepime, ceftazidime, meropenem monotherapy and ceftazidime plus amikacin in febrile neutropenia patients have been comparable ranging from 52 to 56%. The Infectious Disease Society of America

Table 3 Empirical antifungal therapy
Tabela 3. *Empirijska antifungalna terapija*
EMPIRICAL ANTIFUNGAL THERAPY
The optimal time to start antifungal therapy depends on the degree of risk for fungal infection, generally 4-7 days after the onset of FN, despite the use of broad-spectrum antibiotics. Any further delay in the empiric administration of an antifungal drug leads to a significant increase in the mortality rate in high-risk patients
EMPIRIJSKA ANTIGLJIVIČNA TERAPIJA
Optimalno vreme za započinjanje antigljivične terapije zavisi od stepena rizika za razvoj gljivične infekcije i obično iznosi 4-7 dana nakon početka febrilne neutropenije uprkos primeni antibiotika širokog sprktra. Svako dalje odlaganje započinjanja empirijske antigljivične terapije dovodi do značajnog porasta mortaliteta kod visokorizičnih pacijenata.

ANTIFUNGAL DRUGS FOR EMPIRICAL THERAPY (ECIL-6 2017)
In neutropenic patients with persistent fever despite broad-spectrum antibiotic therapy
EMPRIJSKA ANTIGLJIVIČNA TERAPIJA
Kod neutropeničnih pacijenata sa perzistentno povišenom telesnom temperaturom i pored primenjene antibiotske terapije širokog spektra

Antifungal drug *Antigljivični lekovi*	Daily dose *Dnevna doza*	CDC scale/*CDC gradacija*		
		Recommendation *Preporuka*	Effectiveness *Efikasnost*	Safety *Sigurnost*
Amphotericin B liposomal	3 mg/kg	A	I	I
Caspofungin	50 mg	A	I	I
Amphotericin B colloidal dispersion	4 mg/kg	B	I	I
Amphotericin B lipid complex	5 mg/kg	B	I	I
Voriconazole	2x3 mg/kg iv.	B	I	I
Amphotericin B Deoxycholate	0,5-1 mg/kg	B/D	I	I
Itraconazole	200 mg	C	I	I
Fluconazole	400 mg iv.	C	I	I
Micafungin	100mg iv.	B	II	II

(IDSA) guidelines now support the use of agents such as ceftazidime, cefepime, imipenem, and meropenem as alternatives for monotherapy. However, combination therapy seems to be more effective in patients with documented gram-negative bacillary bacteremia and may be associated with a lower rate of initial empirical treatment modification and shorter duration [3, 4, 6, 7].

Empirical antifungal therapy

Neutropenic patients who remain febrile despite 4–7 days of broad-spectrum antibacterial therapy are at a high risk of invasive fungal infection [8]. Empirical antifungal therapy is defined as the institution of antifungal treatment in persistently febrile neutropenic patients and other high-risk patients. In two small-randomized studies in the 1980s amphotericin B was shown to reduce the risk of invasive fungal infection by 50–80% and the risk of fungal infection related mortality by 23–45%. The IDSA has recommended that amphotericin B at 0.5– 0.7 mg/kg/day be administered till marrow recovery [8–10]. This approach is limited however by the adverse effects caused by drug infusion (fever, chills, myalgias, nausea, hypotension and bronchospasm). Lipid formulations which improve the therapeutic ratio of the traditional formulation are available: amphotericin B in lipid complex (ABCL), amphotericin B colloid dispersion (ABCD), liposomal amphotericin B (Ambisome) and Indian liposomal amphotericin B (Fungisome). The safety and efficacy of these formulations are well established. These formulations have comparable efficacy and are less nephrotoxic than conventional amphotericin B, however their usage is limited by the high cost [11, 12]. Comparative studies have shown that all of the lipid formulations are effective to comparable degrees that liposomal amphotericin B is the least toxic and lower doses (1–3 mg/kg/day) are as effective as higher doses (5 mg/kg/dayA neutropenic fever showed liposomal amphotericin B to be associated with fewer break through fungal infections, less infusion related toxicity and less nephrotoxicity as compared to amphotericin B deoxycholate [11, 12]. More recently, intravenous itraconazole, a triazole with activity against both molds and yeasts, has been shown to be equivalent to amphotericin B. In view of the limited activity of fluconazole against Aspergillus species and some non-albicans Candida species, patients with documented invasive fungal infections should not be treated with this drug. Results of trials assessing the activity of voriconazole, a new azole and capsofungin, a new candin, in the treatment of invasive fungal infections are encouraging **(Table 3)**. A recently published study compared voriconazole to a lipid preparation of amphotericin B in the empirical treatment of febrile neutropenia [9–12]. This was an open labeled, randomized study with a non-inferiority design. The overall success rate was 26% with voriconazole and 30.6% with liposomal amphotericin B. No statistical significance was observed. However there were fewer documented breakthrough infections

with voriconazole as compared to liposomal amphotericin B. (5.3 vs. 1.2%). The voriconazole group had fewer cases of severe infusion related reactions ($P < 0.01$) and of nephrotoxicity ($P < 0.001$). The incidence of hepatotoxicity was similar in the two groups. In another trial comparing voriconazole with amphotericin B deoxycholate in documented invasive Aspergillus infection, voriconazole was associated with a response rate of 52.8 vs. 31.6% for amphotericin B [9–14]. In the intention to treat analysis, the 12 week overall survival in the voriconazole group was 70.8 vs. 57.9% in the amphotericin arm. Unfortunately, the greater cost of therapy of the lipid formulations limits their broader utilization as less toxic alternatives to conventional amphotericin B. The choice of antifungal agent is a critical issue among high-risk neutropenic patients and hematopoietic stem cell transplant patients. Such patients often receive concomitant nephrotoxic drugs and have pre-existing renal impairment or diminished renal reserve. A lipid formulation of amphotericin B is appropriate as initial empirical therapy or as definitive therapy for proven mycosis in high-risk patients receiving concomitant nephrotoxic drugs (cyclosporine), those with pre-existing renal impairment and those with protracted neutropenia during which dose limiting toxicity may occur [9–14].

Conclusion

Bearing in mind that serious infections in patients with hematological malignancies are the main cause of morbidity and mortality, it is necessary to further develop a strategy for the prevention and treatment of febrile neutropenia.

Prompt application of empiric antibiotic and antifungal therapy is crucial for the outcome of the treatment of these high-risk hematological patients. The teamwork of hematologists, microbiologists, infectious disease specialists, epidemiologists, and radiologists is extremely important.

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Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al.*

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG, GIF, TIFF, EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.