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SADRŽAJ

ORIGINAL STUDIES
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University of Novi Sad, Faculty of Medicine[1]
Institute for Child and Youth Health Care of Vojvodina, Novi Sad[2]

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EFFICIENCY OF ULTRASOUND GUIDED LOWER LIMB PERIPHERAL NERVE BLOCKS IN PERIOPERATIVE PAIN MANAGEMENT FOR KNEE ARTHROSCOPY IN CHILDREN. A RANDOMIZED STUDY

EFIKASNOST ULTRAZVUKOM VOĐENIH PERIFERNIH NERVNIH BLOKOVA DONJEG EKSTREMITETA U PERIOPERATIVNOJ ANALGEZIJI ZA ARTROSKOPIJU KOLENA KOD DECE. RANDOMIZOVANA STUDIJA

Dragan MARINKOVIĆ[1,2], Jovana M. SIMIN[2], Biljana DRAŠKOVIĆ[1,2], Ivana M. KVRGIĆ[2] and Marina PANDUROV[2]

Summary

Introduction. Ultrasound guided lower limb peripheral nerve blocks are efficient for perioperative pain treatment in children. The aim was to see if lower limb peripheral nerve blocks reduced the amount of propofol and opioid analgesics used intraoperatively, as well as the level of pain and consumption of systemic analgesics postoperatively. **Material and Methods.** A randomized, prospective clinical trial was carried out. It included 60 children between 11 and 18 years of age scheduled for elective knee arthroscopy. The patients were divided into two groups. Group A received general anesthesia, group B received lower limb peripheral nerve blocks with sedation or general anesthesia. Postoperative level of pain was assessed using visual analogue scale. **Results.** Less propofol and fentanyl was used to induce and maintain anesthesia in group B ($p<0.001$). The level of postoperative pain was significantly lower in group B ($p<0.001$), as well as the postoperative consumption of analgesics ($p<0.001$). As mahbny as 47% of the patients were discharged without receiving any analgesics postoperatively. The average duration of peripheral nerve blocks was 468 minutes. **Conclusions.** Ultrasound guided lower limb peripheral nerve blocks are an efficient technique of regional anesthesia in children. They reduce the amount of general anesthetics and opioid analgesics needed intraoperatively as well as the level of postoperative pain and consumption of analgesics postoperatively.

Key words: Nerve Block; Ultrasonography, Interventional; Peripheral Nerves; Lower Extremity; Arthroscopy; Knee Joint; Perioperative Care; Pain Management; Pain Measurement; Child; Anesthetics; Analgesia; Anesthesia, Conduction; Anesthesia, General

Sažetak

Uvod. Ultrazvukom vođeni periferni nervni blokovi donjih ekstremiteta efikasna su tehnika perioperativne terapije bola kod dece. Cilj ovog istraživanja bio je da se utvrdi da li periferni nervni blokovi donjih ekstremiteta smanjuju potrošnju opštih anestetika, opioidnih analgetika i mišićnih relaksanata intraoperativno, kao i da li značajno utiču na jačinu bola i potrošnju sistemskih analgetika postoperativno. **Materijal i metode.** Sprovedeno je randomizovano, prospektivno kliničko istraživanje koje je obuhvatilo 60 dece uzrasta od 11 do 18 godina kod kojih je urađena elektivna artroskopija kolena. Pacijenti su podeljeni u dve grupe. Grupu A činili su pacijenti koji su podvrgnuti opštoj, a grupu B pacijenti koji su dobili periferne nervne blokove donjeg ekstremiteta, uz sedaciju ili opštu anesteziju. Jačina postoperativnog bola procenjivana je vizuelno-analognom skalom. **Rezultati.** U grupi B zabeležena je manja prosečna potrošnja propofola i fentanila za uvod i održavanje anestezije ($p<0,001$). Nivo postoperativnog bola bio je statistički značajno niži u grupi B ($p< 0,001$), kao i postoperativna primena sistemskih analgetika ($p<0,001$). Čak 47% pacijenata otpušteno je iz bolnice bez potrebe za dodatnom analgezijom postoperativno. Prosečno trajanje perifernih nervnih blokova bilo je 468 minuta. **Zaključak.** Ultrazvukom vođeni periferni nervni blokovi donjih ekstremiteta predstavljaju efikasnu tehniku regionalne anestezije u dečjem uzrastu. Oni smanjuju količinu opštih anestetika i opiodnih analgetika potrebnih intraoperativno, kao i jačinu bola i potrošnju analgetika postoperativno.

Ključne reči: Nervni blok; Interventni ultrazvuk; Periferni nervi; Donji ekstremiteti; Artroskopija; Zglob kolena; Perioperativna nega; Terapija bola; Procena bola; Dete; Anestetici; Analgetici; Regionalna anestezija; Opšta anestezija

Introduction

Regional anesthesia, especially peripheral nerve blocks (PNB), is increasingly used in the perioperative pain treatment [1]. Introduction of the ultrasound (US) guided technique is responsible for the increasing use of PNB in pediatric population [2]. The US guided technique has enabled better orientation, visualization of the needle and the spreading of the local anesthetic (LA) around nerves, resulting

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Abbreviations

PNB – peripheral nerve blocks
VAS – visual analogue scale
US – ultrasound
LA – local anesthetic
ASA – American Society of Anesthesiologist

in increased precision and reduced complications. In addition, it reduces the unpleasant feeling associated with the landmark-nerve stimulator technique and therefore increases the patient's satisfaction. Furthermore, this technique reduces the time needed to perform PNB and the time to its onset; it increases the intensity and duration of sensory block, and last but not least, it reduces the amount of the LA used and the cost of treatment. US guided PNB also reduce the need for general anesthetics and analgesics, their adverse reactions and complications, leading to faster postoperative recovery and shorter hospital stay [3, 4].

The aim of the research was to see whether US guided lower limb PNB reduced the amount of propofol and opioid analgesics used intraoperatively as well as the intensity of pain and consumption of systemic analgesics postoperatively.

Material and Methods

This randomized prospective clinical research was carried out at the Clinic for Pediatric Surgery, at the Institute for Child and Youth Healthcare of Vojvodina, in Novi Sad. Sixty patients admitted for scheduled knee arthroscopy, between 11 and 18 years of age, were randomized into two groups by flipping a coin. Group A received general anesthesia, while group B patients were given US guided lower limb PNBs with general anesthesia or sedation. Some group B patients received femoral nerve block with obturator or ischiadic nerve block, or both of them.

All patients admitted for elective knee arthroscopy during the observed period of time were included in the study. The exclusion criteria were parents' objection, allergy to a local anesthetic, hemorrhagic diathesis, and neurological deficit of the lower limb on which PNB was supposed to be performed.

All children underwent preoperative and psychological preparation one day before the surgery, accompanied by a parent. All patients were ASA I and ASA II category according to the American Society of Anesthesiologist (ASA). After premedication with oral midazolam (Dormicum®, Rosche) 0,5 mg/kg (maximum dose 15 mg) 45 minutes before the operation, the peripheral venous cannula was placed.

Group A underwent standard monitoring (pulse oximetry, electrocardiography, non-invasive arterial pressure and capnography) and general anesthesia was induced with fentanyl (Fentanyl-Janssen®, Janssen-Cilag, 50 μg/ml) 1 μg/kg, propofol (Propofol 1%®, Fresenius) 2-3 mg/kg and rocuronium (Esmeron®, Schering-Plough, 50 mg/5 ml) 0,6 mg/kg. Subsequently, the laryngeal mask was placed (LMA, Intersurgical) and general anesthesia was maintained with continuous infusion of propofol (6-10 mg/kg/h), along with bolus doses of fentanyl (1 μg/kg) and rocuronium (0, 15 mg/kg) and inhalation of air/O_2 mixture (FiO_2 50%). The lungs were mechanically ventilated using a pressure-controlled mode to maintain $EtCO_2$ between 4.7 and 5.3 kPa.

In group B, US guided lower limb PNBs were performed with sedation or under general anesthesia, depending on the child's age and the level of cooperation. For sedation, 0.1 mg/kg intravenous midazolam (Dormicum® Rosche, 15 mg/ml) was used. In the patients requiring general anesthesia it was conducted in the same manner as in group A, without the administration of muscle relaxants. After the orientation and visualization of nerve structures, 0.25% or 0.33% levobupivacaine (Chirocaine 5 mg/ml, Abbott) 1 ml/kg (2.5-3 mg/kg) was administered under direct ultrasonographic guidance with 'in-plane' technique and a 22G (Stimuplex®D, Braun) needle. The puncture area and the ultrasound probe were prepared in a sterile manner. The puncture site was protected and the operation began 20 minutes later (**Figure 1**).

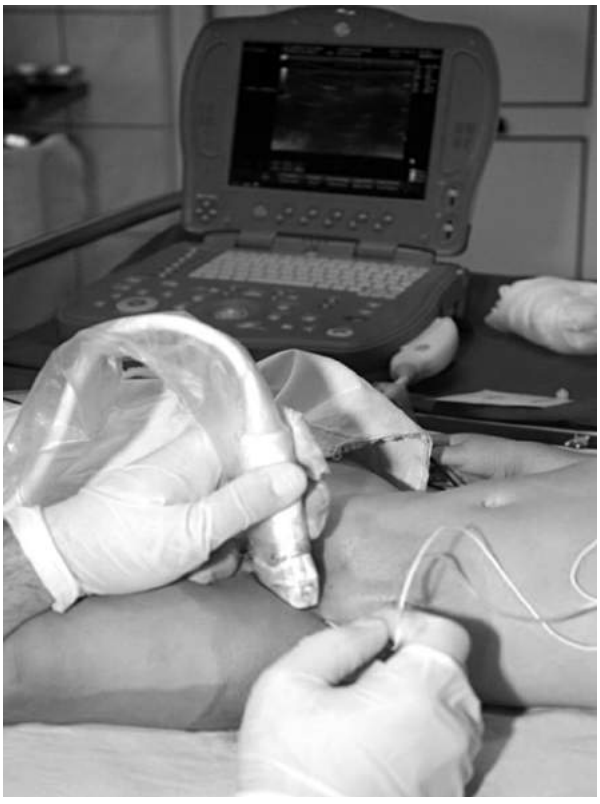


Figure 1. US guided femoral nerve block.
Slika 1. *Izvođenje bloka femoralnog nerva pod kontrolom ultrazvuka*

Vital parameters as well as the overall consumption of anesthetics and opioid analgesics were recorded during the operation. The level of postoperative pain was assessed using Wong Baker FACES scale or visual-

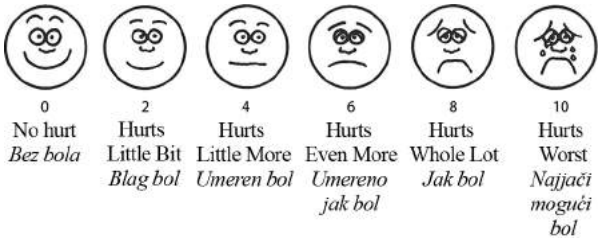


Figure 2. Wong Baker FACES scale
Slika 2. *Wong Baker FACES skala*

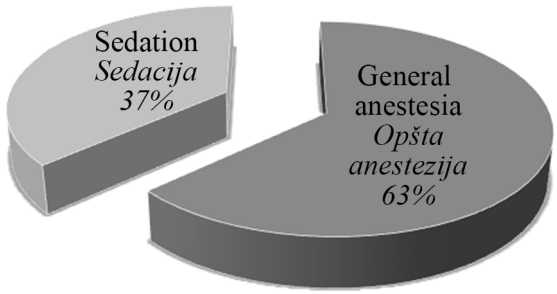
analogue scale (VAS) right after the operation and 2, 6 and 12 hours after the operation. The pain level was marked with 0 to 10 grades, 0 being the lowest and 10 the highest pain level (**Figure 2**). The time to the first dose of systemic analgesic, overall analgesic consumption and adverse events was also recorded.

Statistical analysis was done using SPSS package 13.0 for Windows. The statistical significance between the average values of parametric data was tested using Student's t-test, and χ^2 test was used for non-parametric data. P value below 0.05 was considered statistically significant.

Results

Sixty patients were included in the study, thirty in each group. No statistically significant differences were calculated between the study groups regarding the age, weight, sex or the duration of surgery (**Table 1**).

Some of the group B patients received general anesthesia in addition to the PNB, while others were sedated (**Graph 1**).



Graph 1. General anesthesia or sedation in group B
Grafikon 1. *Procentualna zastupljenost opšte anestezije i sedacije u grupi B*

Intraoperative consumption of propofol and fentanyl is presented in **Table 2**, while different combinations of lower limb PNB used are shown in **Table 3**. Group B patients did not receive any muscle relaxants during general anesthesia.

During the performance of PNB, 80% of patients were sedated, while the rest were under general anesthesia. In 47% of cases, the US guided lower limb PNB was conducted without the addition of nerve stimulator.

The average PNB duration was defined as the time from the administration of PNB to the administration of the first dose of systemic analgesic given to relieve the pain. The average length of PNB was 468 minutes (100-1290 minutes).

The pain level assessed at different time points is presented in **Table 4**. Whereas all group A patients needed analgesics postoperatively, only 47% of group

Table 1. Patients' characteristics in both groups. Data are presented as mean values (SD).
Tabela 1. *Osobine pacijenata po grupama. Podaci su prikazani kao srednje vrednosti (SD).*

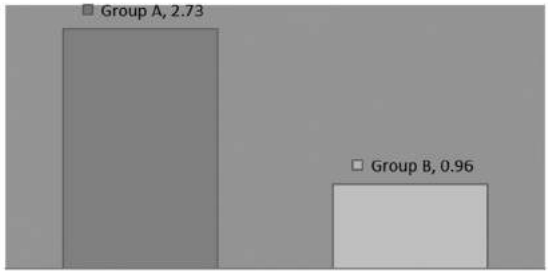
	Group A/*Grupa A*	Group B/*Grupa B*	p value/*p-vrednost*
Age (years)/*Starost (godine)*	14.53 (3.19)	15.2 (1.57)	p>0.001
Weight/*Masa* (kg)	61.55 (17.93)	64.35 (16.15)	p>0.001
Duration of operation/Trajanje operacije (min)	64.5 (21.66)	63.66 (21.53)	p>0.001

Table 2. Intraoperative consumption of propofol and fentanyl, mean (SD)
Tabela 2. *Intraoperativna potrošnja propofola i fentanila, srednja vrednost (SD*

	Group A/*Grupa A*	Group B/*Grupa B*	p value/*p-vrednost*
Induction propofol/*Propofol za uvod* (mg/kg)	2.26 (0.33)	1.59 (0.97)	p<0.001
Maintainance propofol/*Propofol za održavanje* (mg/kg/h)	6.45 (1.81)	4.18 (1.20)	p<0.001
Fentanyl/*Fentanil* (μg/kg/h)	2.12 (0.68)	1.13 (0.53)	p<0.001

Table 3. Types of PNBs performed
Tabela 3. *Korišćeni periferni nervni blokovi*

Type of block/*Vrsta bloka*	Number of patients/*Broj pacijenata* (%)
Femoral, obturator, ischiadic/*Femoralni, opturatorni, išijadični*	13 (43.33)
Femoral, obturator/*Femoralni, opturatorni*	10 (33.33)
Femoral, ischiadic/*Femoralni, išijadični*	7 (23.33)



Graph 2. Average postoperative analgesics demand (p<0.001).
Grafikon 2. *Prosečna postoperativna potrošnja analgetika (p < 0,001)*

B patients required postoperative analgesia. Others were discharged without receiving any analgesics postoperatively. The average postoperative analgesics demand is presented in **Graph 2**. No side-effects related to anesthesia technique were recorded.

Discussion

Regional anesthesia, especially PNBs, is increasingly used in the perioperative pain treatment [1]. The introduction of the US guided technique is responsible for the increasing use of PNB in pediatric population [2]. In our country this technique was not used in everyday clinical practice until a couple of years ago. Our Clinic is one of the first institutions where it gained significant popularity.

In our study the precise administration of local anesthetic under the ultrasound guidance to group B patients resulted in efficient lower limb PNB, with no complications recorded. Knee arthroscopy was done under sedation only in as many as 37% of these patients. By reducing the need for general anesthesia, one can expect better hemodynamic stability, reduction of perioperative stress response and fewer complications [5, 6].

The amount of propofol given intraoperatively to induce and maintain anesthesia was significantly lower in group B patients who received the combination of PNB and general anesthesia. This is of utmost importance since there is growing and convincing evidence that the exposure to anesthetics in common clinical practice can be neurotoxic to the developing brain and lead to long-term neurological sequelae [7–9].

There was significantly lower intraoperative demand for opioid analgesics in group B, meaning that the lower limb PNB provided the adequate intraoperative analgesia. The reduced need for opioids leads to its reduced side-effects such as postoperative nausea and vomiting, pruritus and respiratory depression. Our results are in accordance with other studies which examined the efficacy of PNBs in knee surgery [10–13].

Muscle relaxant, rocuronium, was used only in group A patients since PNB provided adequate muscle relaxation in group B. The reduced need for muscle relaxants decreases the possibility of adverse drug reactions, such as histamine liberation or allergic reactions, ranging from rash to anaphylaxis [14–16].

During the performance of PNB, 80% of patients were sedated, while others were under general anesthesia, which is in accordance with the findings of other authors [2].

In 47% of cases, the US guided PNB was conducted without the addition of a nerve stimulator, which made the overall experience of administration of local anesthetic much less unpleasant. Marhofer et al. stressed the importance of the US guided technique in the reduction of pain sensation caused by muscle contractions and repeated needle positioning [1, 2]. Due to the precise needle visualization there is no need for multiple needle adjustments and hence the sensation of pain is reduced.

The average PNB duration was defined as the time from the administration of PNB to the administration of the first dose of systemic analgesic given to relieve the pain, as found in the literature [2, 17]. The average length of lower limb PNB was 468 minutes (100-1290 minutes), which is in accordance with the results of Marhofer [2] and Oberndorfer et al. [17].

In our study, 0.25% and 0.33% levobupivacaine was used for lower limb PNBs, the average dose being 1.75 mg/kg. These results are in accordance with the findings of other authors who have proved that lower doses can be used for PNB due to precise visualization of the needle and the spreading of the LA [17–24]. This is particularly relevant for neonates and infants who are at risk of local anesthetic toxicity and higher free plasma concentrations of local anesthetic agents in view of their lower plasma concentration of the binding protein alpha-1acid glycoprotein [25].

The level of postoperative pain assessed with Wong Backer FACES scale and VAS was significantly lower in group B. Consequently, the average number of doses of systemic analgesics administered postoperatively was significantly lower in this group of patients. It is interesting to point out that

Table 4. Pain level assessment at different time points after operation, mean (SD)
Tabela 4. *Procena jačine bola u određenim vremenskim intervalima nakon operacije, srednja vrednost (SD)*

Pain level/*Nivo bola*	Group A/*Grupa A*	Group B/*Grupa B*	p value/*p vrednost*
Initially/*Na početku*	2.8 (1.34)	0.4 (0.81)	p<0.001
After 2 h/*Posle 2 h*	4.73 (1.52)	0.66 (1.32)	p<0.001
After 6 h/*Posle 6 h*	.73 (0.69)	.20 (1.78)	p<0.001
After 12 h/*Posle 12 h*	5.93 (0.36)	1.90 (2.00)	p<0.001

as many as 47% of these patients were discharged without the need for any analgesics postoperatively. The same results were observed by Gonzalaes et al [26] and Edkin et al [27], who have concluded that PNB are a good technique of postoperative pain management for knee arthroscopy.

No complications were recorded either during PNB performance or postoperatively in our study. The use of US reduces the incidence of possible complications associated with PNB performed using a landmark and nerve stimulator technique [25, 28].

Conclusion

The ultrasound guided peripheral nerve block is a safe technique of regional anesthesia in children. The combination of regional and general anesthesia reduces the consumption of general anesthetics, opioid analgesics and muscle relaxants, and hence their side effects and possible complications. In addition, the ultrasound guided peripheral nerve blocks reduce the level of postoperative pain and consumption of analgesics postoperatively.

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DRUG UTILIZATION IN TREATMENT OF THYROID DISORDERS DURING PREGNANCY IN SERBIA

UPOTREBA LEKOVA U LEČENJU BOLESTI ŠTITASTE ŽLEZDE TOKOM TRUDNOĆE U SRBIJI

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Summary

Introduction. Depleted uranium radiation and pollution with polychlorinated biphenyls resulting from bombings the territories of Serbia as well as the additional long-term stress may have affected the function of thyroid gland. The objective of this study was to determine the trend of drug utilization in the treatment of thyroid dysfunction during pregnancy in Novi Sad. **Material and Methods.** Women who had given birth at the Department of Gynecology in 1989, 1999, 2007 and 2011 were interviewed during a one-month period about thyroid diseases in the pregnancy as well as the drugs they had taken. **Results.** Not a single pregnant woman was reported to have a thyroid disorder in 1989 and 1999, while in 2007 four women were reported to have a thyroid dysfunction. In 2011, fourteen out of 18 women with thyroid dysfunction were using levothyroxine and in most cases hypothyroidism was diagnosed as autoimmune Hashimoto thyroiditis. **Conclusion.** The study results suggest the necessity of performing more detailed analyses of the correlation between the frequency of the thyroid gland dysfunction and the effects of environmental pollution in Serbia.

Key words: Thyroid Diseases; Pregnancy; Hashimoto Disease; Hypothyroidism; Propylthiouracil; Thyroxine; Risk Factors; Drug Therapy; Epidemiology; Serbia

Sažetak

Uvod. Od zagađujućih supstancija, kao rezultat konfliktnih situacija na području Srbije, značajno mesto zauzimaju osiromašeni uranijum i polihlorovani bifenili koji, uz propratni dugotrajni stres, značajno utiču na funkciju štitaste žlezde. Cilj rada bio je da se odredi trend upotrebe lekova u terapiji bolesti štitaste žlezde tokom trudnoće na teritoriji grada Novog Sada. **Materijal i metode.** Tokom jednomesečnog perioda anketirane su sve porodilje hospitalizovane na Klinici za ginekologiju i akušerstvo 1989, 1999, 2007. i 2011. godine, o bolestima štitaste žlezde, kao i o tome koji lek uzimaju u terapiji poremećaja funkcije štitaste žlezde. **Rezultati.** Tokom 1989. i 1999. godine nijedna trudnica nije evidentirana sa poremećajem funkcije štitaste žlezde, 2007. godine evidentirane su 4 trudnice, a 2011. godine bilo je 18 trudnica sa poremećajem funkcije štitaste žlezde od čega čak 14 sa dijagnozom hipotiroze i to u najvećem broju slučaja kao autoimuni Hašimotov tiroiditis. **Zaključak.** Rezulati našeg rada ukazuju na neophodnost sprovođenja detaljnije analize povezanosti učestalosti ovih poremećaja funkcije štitaste žlezde i uticaja zagađivača životne sredine.

Ključne reči: Oboljenja štitne žlezde; Trudnoća; Hašimotova bolest; Hipotireoza; Propiltiouracil; Tiroksin; Faktori rizika; Terapija; Epidemiologija; Srbija

Introduction

A wide variety of environmental agents affect the thyroid gland causing different thyroid function disorders and one of the well-known environmental catastrophes was the Chernobyl accident, when a significant correlation between the exposure to radioactive iodine J^{131} and the incidence of hypothyroidism was determined [1, 2]. This catastrophe led to the increased frequency of juvenile hypothyroidism in Belarus, the Russian Federation and Ukraine ten years later [3].

During the end stage of conflict period in the Balkans (in 1999), a partial or total destruction of industrial plants, military targets, infrastructure, as well as uncontrolled fires and explosions resulted in large amounts of hazardous organic matter that were generated and emitted into the environment, and the city of Novi Sad was recognized as an environmental hot spot by the United Nations Environment Programme and the United Nations Centre for Human Settlements [4]. About 73,000 tons of crude oil and oil products, including the transformer oil containing polychlorinated biphenyls (PCBs) were burned or leaked (UNEP/UNCHS Report, 1999) during the bombing of the Novi Sad oil refinery in 1999 [5]. The effect of PBC exposure on serum thyroid hormone levels is well documented in animals and humans [5–8]. Some of the studies have shown that the increased prevalence of some

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Abbreviations
PCBs – polychlorinated biphenyls
DU – depleted uranium
DUP – drug utilization during pregnancy and puerperium
TSH – thyroid-stimulating hormone

thyroid antibodies may be related to the known immunomodulatory effects of PCBs [9, 10]. Depleted uranium (DU), an emerging environmental pollutant, was used as armor-penetrating ammunition in the Balkans conflict and was claimed to contribute to health problems [11, 12]. Studies investigating DU health effects at the cellular level or on animals exposed to DU suggest a possible influence on birth defects, immune system impairments, cancer risk etc. [13, 14].

Maintenance of normal thyroid function is essential for psychological and physiological well-being. Pregnant women with undiagnosed or inadequately treated hypothyroidism have an increased risk of miscarriage, preterm delivery, and severe developmental problems in their children [15, 16]. Therefore, the aim of our study was to determine the trend of thyroid function disorders through monitoring the utilization of drugs for the treatment of thyroid gland disorders in pregnant women, as the most vulnerable group of population in the city of Novi Sad, Serbia, before and after the conflict period in the Balkans.

Material and Methods

A survey was performed in Novi Sad, Serbia, a city with about 300,000 inhabitants, with only one Department of Gynecology and Obstetrics. The Regional Ethics Committee approved the survey. The study sample consisting only of women who had signed the informed consent form, thus showing that they were willing to participate in the survey.

This retrospective-prospective study included the pregnant women who had given birth at the Ward of Perinatology, Department of Gynecology and Obstetrics during the one month period in 1989, 1999, 2007 and 2011. The study was the extension of multinational DUP study (Drug utilization during pregnancy and puerperium) [17, 18].

Data about drugs used for thyroid function disorders during pregnancy were taken from the health files of parturients.

Results

Table 1 shows the trend in the drug utilization in treatment of thyroid dysfunction in pregnant women from 1988 to 2011 in Novi Sad.

In years 1988 and 1999 no drugs were used for treatment of thyroid dysfunction in pregnant women included in the surveys (296 and 100 pregnant women, respectively).

In 2007, four out of 423 pregnant women included in the study were reported to have thyroid dysfunction. Three (0.70%) out of four had hypothyroidism and all of them were administered levothyroxine replacement therapy, while one pregnant woman had hyperthyroidism.

In 2011, 18 out of 413 pregnant women included in the study were reported to have thyroid dysfuncti-

Table 1. The prevalence of the thyroid dysfunction in pregnant women suffering from hypothyroidism and hyperthyroidism included in the survey at the Ward of Perinatology, Department of Gynecology and Obstetrics, Clinical Center Novi Sad in 1988, 1999, 2007 and 2011.
Tabela 1. *Prevalencija poremećaja funkcije štitaste žlezde kod trudnica obolelih od hipotiroze i hipertiroze uključenih u ispitivanje na Zavodu za perinatologiju, Klinike za Ginekologiju i akušerstvo Kliničkog centra u Novom Sadu 1988., 1999., 2007. i 2011. godine.*

	1989*	1999**	2007**	2011
Total No of included pregnant women *Ukupan broj porodilja uključenih u ispitivanje*	296	100	423	413
Total No and percentage of pregnant women with thyroid gland disorders *Ukupan broj i procenat porodilja sa poremećajem funkcije štitne žlezde*	0	0	4 (0.93%)	18 (4.23%)
Total No and percentage of pregnant women with hypothyroidism *Ukupan broj i procenat porodilja hipotireozom*	0	0	3 (0.70%)	14 (3.39%)
Total No and percentage of pregnant women with hyperthyroidism *Ukupan broj i procenat porodilja hipertireozom*	0	0	1 (0.23%)	4 (0.97%)

* data about incidence of the thyroid disorders were taken from the Study on Drug Use in Pregnancy (DUP) initiated by the Mario Negri Institute, Milano, Italy, conducted in 1989, when Novi Sad was one of the centres of the study. The follow-up study was performed 10 years later in 1999 (Sabo et al., 2001)

** data about incidence of the thyroid disorders were taken from the unpublished data of the Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad

* *podaci o učestalosti oboljenja štitaste žlezde kod trudnica preuzeti su iz istraživanja o upotrebi lekova u trudnoći –DUP (Drug Use in Pregnancy) koji je pokrenuo Institut Mario Negri, Milano, Italija, sprovedenog 1989. godine kada je Novi Sad bio jedan od centara ovog istraživanja. Nastavak ovog istraživanja sproveden je 10 godina kasnije 1999. godine [17]*

** *podaci o učestalosti oboljenja štitaste žlezde kod trudnica preuzeti su iz neobjavljenih podataka Katedre za farmakologiju, toksikologiju i kliničku farmakologiju*

on. The replacement therapy of levothyroxine for the treatment of hypothyroidism was taken by 14 (3.39%) women during pregnancy out of 413 women included in the study, with the average duration of the treatment with levothyroxine sodium of 3.3±2.59 years. Autoimmune Hashimoto's thyroiditis was diagnosed in 64.28% of them. The average duration of treatment with propylthiouracil was 1.6±0.94 years in four pregnant women with hyperthyroidism.

Discussion

Thyroid disorders are the second most common endocrine disorders found in pregnancy. However, data on epidemiology of thyroid diseases are scarce [19–21]. The epidemiology data vary widely, with overt hypothyroidism occurring in 0.3–0.5% [22] to 12.8% of pregnancies [22, 23]. Subclinical disorders appear to occur from 2–3% to 25% [21–24]. Therefore, the more accurate way of assessing the epidemiology of thyroid dysfunction is to follow the prevalence in time rather than to compare epidemiology between the countries.

In Novi Sad study the percentage of pregnant women taking drugs for hormonal disorders increased more than 4-fold between the last two study periods. Having in mind the significance of hypothyroidism on fetal development, such a high increase in the number of young pregnant women with hypothyroidism is alarming.

According to the published data, numerous reasons can trigger the autoimmune process affecting the thyroid gland, chemical factors and long-term stress being well recognized. The ammunition containing depleted uranium was used in military operations by NATO in all parts of Serbia during the Kosovo conflict in 1999. Public concern related to health consequences has been raised by the general belief that Novi Sad and the region of Vojvodina were contaminated by depleted uranium during the bombing. Some studies in animals have shown the immune modulation ability of DU. It can cause inappropriate apoptosis of murine (mouse) peritoneal macrophages, which can lead to both autoimmune problems and immusuppression. The similarities between murine and human immune system genetics suggest that these findings may also apply to humans [25]. The analysis of 50 soil samples taken from the region of Vojvodina after the conflict showed slight increases in the activity of 137Cs, which has a negative impact on the environment, from 6.6 in 1989 to 8.2 Bq/kg in 2001 in the region of Novi Sad and from 23 to 31Bq/kg in the region of Vršac [26]. Most of 137Cs originates from Chernobyl disaster [27]. However, while the values in Novi Sad and Vršac slightly increased after 1999, in Subotica region which had not been bombed, with time the activity of 137Cs had the tendency to decline from 9Bq/kg in 1999 to 4,6Bq/kg in 2001 year. Contamination with inhaled DU was proved by the excretion of DU isotopes in the urine of exposed military personnel seven years after the Balkans conflict according to Durakovic (2001) [11].

Škrbić and Miljević investigated the soil pollution at the oil refinery in Novi Sad following the destruction of crude oil and its products in storage tanks during the Kosovo conflict by taking the soil samples from several fields of the oil refinery [28]. At some locations the presence of PCBs, another important environment pollutant, indicated the burning of additives stored at the refinery site. The contamination with PCBs presents a great environmental risk, especially for drinking water (the oil refinery of Novi Sad is located just 0.5 km upstream of bank filtration wells used for the city's water supply) due to the potential migration of identified pollutants by groundwater to infiltration galleries [29]. In adults, adolescents and children from highly PCB-exposed areas the concentration of PCB in blood samples correlated negatively to the levels of circulating peripheral thyroid hormones [30]. The levels of PCBs in the environment are associated with reduced thyroid hormone levels in the pregnant women [31, 32]. Prenatal or postnatal exposure of humans or animals to PBCs can result in hormonal changes and neurodevelopment deficits [33, 34]. The long-term exposure to heavy environment pollution among the employees of PBCs factory in Slovakia resulted in a significant increase of autoantibodies against thyroid peroxidase (TPO Ab) as well as a higher prevalence of autoantibodies against thyroglobulin (Tg Ab) and autoantibodies against TSH (TSHR Ab) especially in female employees compared to the control group [35].

Numerous other reasons can affect the prevalence of dysfunction of thyroid gland, long-lasting stress being one of them [36]. Studies including soldiers after the war and victims of abuse have shown abnormal values of TSH, triiodthyromine (T3) and thyroxine (T4), with mostly decreased values of thyroid hormones. The effects of chronic stress during the civil war in Serbia have not been investigated yet. Its long lasting consequences in combination with environment pollutants includes not only disturbances of homeostasis, but also a potentially deadly disease such as an increased number of malformations in newborns and different forms of cancer in adults [2, 37, 38].

Limitation of the Study

A shortcoming of this study is the absence of data on the prevalence of thyroid disorders in pregnant women, namely the hypothyroidism, which results from insufficient epidemiology data in Serbia. The translation of results of comparative research of thyroid disorders prevalence is hard to conduct due to differences in definition and samples of population as well as the fact that the use of unique series of diagnostic criteria for the prevalence of these disorders are necessary. The data about the possible causes of environment pollution and stress on hypothyroidism in pregnancy

are scarce, and conclusions about their influence on pregnancy are insufficient so far.

Conclusion

Our study suggests a significant increase of symptomatic autoimmune thyroid disease, namely Hashimoto's thyroiditis, in pregnant women in Novi Sad region during the last decade. Although the reasons have been discussed, they are still not known. Further follow up of this trend and detailed study of possible reasons are necessary. Due to the significance of proper treatment of hypothyroidism in pregnant women, it is recommended to introduce routine testing of all pregnant women in order to diagnose subclinical cases of pregnancy to start treatment on time.

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EXPRESSION OF COLLAGEN TYPE I IN UNALTERED AND OSTEOARTHRITIC MENISCI OF KNEE JOINT

EKSPRESIJA KOLAGENA TIPA I U NEIZMENJENIM I OSTEOARTRITIČNIM MENISKUSIMA ZGLOBA KOLENA ČOVEKA

Igor SLADOJEVIĆ[1], Zdenka KRIVOKUĆA[1], Vesna GAJANIN[1] and Slavko MANOJLOVIĆ[2]

Summary

Introduction. Knee osteoarthritis is a progressive degenerative disease which affects meniscal tissue. The aim of this study was to determine the differences in collagen type I expression in macroscopically unaltered and osteoarthritic menisci, and correlate the expression with the grade of macroscopic damage, age and body mass index of patients, preoperative condition of anterior cruciate ligament, angulation and knee contracture. **Material and Methods**. The control group consisted of 10 macroscopically unaltered menisci, while the experimental group had 35 osteoarthritic menisci. Besides macroscopic grading of meniscal damage, the analysis of collagen type I expression was determined by immunohistochemical staining with the corresponding antibody using semiquantitative scale scores and quantitative parameters: intensity of expression and stained area size. **Results**. The results of semiquantitative evaluation showed a statistically significant decrease in collagen type I expression in osteoarthritic menisci, which correlated with an increase in macroscopic damage grade. The results of quantitative evaluation did not show a statistically significant decrease in the expression. In posterior meniscal horns, a more intense collagen type I expression was seen in the women, as well as a positive correlation of quantitatively evaluated expression with body mass index. Collagen type I expression in the anterior horns was significantly lower in varus alignment. **Conclusion**. In the semiquantitative evaluation, collagen type I expression in osteoarthritic menisci was significantly lower compared to macroscopically unchanged menisci. The decrease in the expression level correlates with the increase in the grade of macroscopic meniscal damage. There was no statistically significant difference in the quantitative evaluation of expression.

Key words: Knee Joint; Menisci, Tibial; Osteoarthritis, Knee; Collagen Type I; Pathological Conditions, Anatomical; Immunohistochemistry

Sažetak

Uvod. Osteoartritis zgloba kolena je progresivna degenerativna bolest u kojoj je zahvaćeno i tkivo meniskusa. Cilj ove studije je utvrđivanje razlika u ekspresiji kolagena tipa I u makroskopski neizmenjenom i osteoartritičnom meniskusu, te koreliracija ekspresije sa stepenom makroskopskog oštećenja, životnom dobu i indeksom telesne mase ispitanika, preoperativnim stanjem prednje ukrštene veze, angulacijom i kontrakturom kolena. **Materijal i metode**. Kontrolnu grupu činilo je 10 makroskopski neizmenjenih, a eksperimentalnu 35 osteoartritičnih meniskusa. Osim makroskopskog stepenovanja oštećenja meniskusa, analiza ekspresije kolagena tipa I vršena je imunohistohemijskim bojenjem odgovarajućim antitelom uz semikvantitativnu skalu skorova i određivanja kvantitativnih parametara: intenziteta imunohistohemijske ekspresije i veličine obojene površine. **Rezultati.** Analizom rezultata semikvantitativne procene nađeno je statistički značajno smanjenje ekspresije kolagena tipa I u osteoartritičnim meniskusima u odnosu na kontrolnu grupu, koje je koreliralo sa porastom stepena makroskopskog oštećenja meniskusa. Analizom rezultata kvantitativne procene, smanjenje ekspresije nije dostiglo nivo statističke značajnosti razlika. U zadnjim rogovima meniskusa registrovana je intenzivnija ekspresija kolagena tipa I kod žena, kao i pozitivna korelacija kvantitativno procenjene ekspresije sa indeksom telesne mase. Ekspresija kolagena tipa I u prednjim rogovima bila je značajno manja kod varusno anguliranih kolena. **Zaključak.** Pri semikvantitativnoj proceni, u osteoartritičnim meniskusima ekspresija kolagena tipa I značajno je niža u odnosu na makroskopski neizmenjene meniskuse. Pad stepena ekspresije u korelaciji je sa porastom stepena makroskopskog oštećenja meniskusa. Pri kvantitativnoj proceni ekspresije, nisu nađene statistički značajne razlike.

Ključne reči: Zglob kolena; Meniskusi; Osteoartritis kolena; Kolagen tip I; Patološka stanja, makroskopski nalaz; Imunohistohemija

Introduction

Menisci are fibrocartilaginous structures of the knee joint, located between femoral condyles and tibial plateau. It is a specialized tissue that plays a role in the transmission of loads, shock absorption, stability of the knee joint and distribution of synovial fluid and nutrients [1]. It is primarily made of interwoven network of collagen fibers, while the cells and the extracellular matrix are inserted between the fibers. The collagen components of menisci have an important functional role in the provision of tissue extensibility [2]. The concentration of collagen in meniscus is higher than in articular cartilage [3]. Collagen type I is dominant in the meniscus (90% of the total collagen content, while the rest of collagen content is

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Abbreviations

OA – osteoarthritis
BMI – body mass index
ACL – anterior cruciate ligament

made of collagen types II, III, IV, V, VI, and X [4]. Collagen types III and V are found in trace amounts (< 1%). The proportion of different types of collagen in the meniscus varies depending on the localization – the peripheral two thirds of the meniscus are made mostly of collagen type I, while the inner third of the meniscus has collagen types II and I in 60%: 40% ratio [5]. In addition to the distribution, the orientation of collagen fibers within the meniscus (circumferential and radial) directly affects the function of the tissue, and it has been observed that the network of collagen fibers in the meniscus changes in the content and structure from one region to another [6, 7].

Osteoarthritis (OA) is a chronic degenerative joint disease that afflicts millions of people worldwide [8]. Due to the expanticipated longer life expectancy, it is estimated that the two-thirds of people over 60 years of age have OA [9]. The knee joint is most frequently affected by osteoartritic changes [10]. Nuclear magnetic resonance (NMR) images of the knee joint have proven that meniscal destruction is one of the characteristics of the late stage of OA of the knee joint [11]. The loss of meniscal function is a potent factor for the development of knee osteoarthritis and cartilage loss in the course of OA [12].

The macroscopic appearance of the meniscus in knee OA depends on the grade of degeneration. Menisci marked as grade 0 have smooth, whitish shiny surface without signs of degeneration, while those with grade 4 have pale color and rough surface with fissures and clefts. In sites of degenerative changes of meniscal tissue, the amount of collagen and noncollagenous proteins is reduced, which decreases the mechanical quality of this tissue [13]. Menisci with a higher degree of degeneration have been found to have significant structural changes, which includes rifts and cysts within the collagen bundles with the formation of cell nests [14].

Collagen fibers in OA menisci become coarser and less organized, and the amount of individual circumferential and radial fibers is significantly reduced [15]. As for the collagen type I, it was found that there is a reduction of its expression in OA menisci, predominantly in the deeper tissue zones [16] and that the expression significantly decreases with the severity of degeneration [17]. A thorough survey of relevant literature has failed to reveal either an analysis of the variability of the expression of collagen type I in relation to the anatomical parts (body, horns) in unaltered and degenerated menisci or any investigations on a possible correlation between the expression and general characteristics of patients and associated changes in joint structure. The aim of this research was to investigate the immunohistochemical expression of collagen type I in unaltered and osteoarthritic menisci of human knee joint, to determine the differences in the expression among the three parts of the meniscus (anterior horn, body, posterior horn) as well as to establish the connections of expression with the gender, age, body mass index (BMI), preoperative contracture, angulation of the knee joint and the condition of anterior cruciate ligament (ACL).

Material and Methods

The research was conducted with the permission of the Ethics committee of the Department of Physical Medicine and Rehabilitation "Dr Miroslav Zotović" in Banja Luka. The informed consent in writing was obtained from every patient or a family member before taking the material. Each participant in the study was given written information with all the details related to the research.

The samples for the study were collected at the Department of Orthopedic Surgery, Department of Physical Medicine and Rehabilitation "Dr Miroslav Zotović" in Banja Luka from 35 consecutive patients who had total knee arthroplasty due to clinically advanced osteoarthritis; thus, 70 menisci (35 medial and 35 lateral) were included in the study sample of which 10 macroscopically unaltered and 35 degenerated menisci were analyzed. The following data were taken from the patients' histories: gender, patient's age, BMI, preoperative contracture, angulation of the knee joint (varus or valgus), preoperative condition of ACL (intact or ruptured) and medications used by the patients.

The study sample did not include patients who had one of the following diagnoses: rheumatoid arthritis, ankylopoetic spondylitis, psoriatic arthritis or reactive arthropathy, as well as those patients who used any chondroprotective drugs, such as corticosteroids, sulfate polysaccharides, chemically modified tetracyclines, diacetylrhein or glucosamine [18], or who had a history of trauma of the knee in which the prosthesis was implanted, and the patients with general systemic weakness or acute contagious disease.

All meniscus samples were placed in the buffered solution of 10% formaldehyde immediately after extraction. After fixation, the obtained meniscus samples were macroscopically analyzed and graded according to the scale of Sun et al. [19]: 0 = normal surface appearance; 1 = minimal fibrillation and degeneration; 2 = moderate fibrillation and degeneration; 3 = severe fibrillation and degeneration, without cleft; 4 = severe fibrillation and degeneration, multiple cleavages, incomplete or complete clefts. Having been graded, the menisci were cut in the frontal plane into 3 levels (the anterior horn, body and posterior horn) and three tissue sections per menisci were made, each 5 mm thick. The tissue sample processing was done according to the ordinary procedure at the Department of Pathology, Clinical Center, Banja Luka. After molding in paraffin, the samples were cut and stained with hematoxylin eosin.

For immunohistochemical analysis of collagen type I, the paraffin tissue blocks were sliced into the semise-

rial sections that were mounted on glass slides (5 sections per a slide). After the standard tissue processing, immunohistochemical procedure was continued by blocking endogenous peroxidase by incubating the tissue in a 3% hydrogen peroxide solution for 10 minutes at room temperature and antigen unmasking in a commercial solution of Proteinase K (Proteinase K ready-to-use, DAKO Corporation, USA) at room temperature for 10 minutes. The incubation was performed immediately prior to the application of primary antibody with commercial Ultra V Block (TA-125-UB, Lab Vision Corporation, Fremont, USA), and then with the corresponding mouse monoclonal anti-collagen I [COL-1] antibody (ab90395, Abcam plc, Cambridge, UK) at a 1: 400 dilution of concentrated antibody. The diluted antibody was poured onto the slides and incubated overnight in a humidified chamber at 4 °C. Antigen unmasking was performed with the detection system made of anti-mouse and anti-rabbit IgG conjugated with horseradish peroxidase (Lab Vision UltraVision LP Detection System: HRP Polymer (Ready-To-Use), Lab Vision Corporation, Fremont, USA) in the first step and DAB chromogen (DAKO liquid DAB + Substrate Chromogen System, DAKO Corporation, USA) in the second step of immunodetection. After washing of chromogen, the tissue sections were contrasted with Maier's hematoxylin.

The evaluation of extracellular expression of the investigated antibody was carried out:

1. according to the following semiquantitative scale: score 0 (absence of staining, the entire extracellular space without staining); score 1 (weak or moderate, focal extracellular positivity/weak diffuse positivity); score 2 (high focal extracellular positivity/moderate diffuse positivity) and score 3 (high diffuse positivity).
2. quantitatively by determining two parameters: the staining intensity score and the surface of cross-sectional area (in μm^2) with the present extracellular expression. This quantification was performed using the software TissueQuant (Manipal Centre for Information Science, Manipal University, India), version 1.0.1.

In order to determine whether a certain knee angulation and the state of ACL together affected collagen type I expression, four subgroups were formed out of the experimental group:

1. Varus angulation with preserved ACL
2. Varus angulation without preserved ACL
3. Valgus angulation with preserved ACL
4. Valgus angulation without preserved ACL, which were used to compare semiquantitatively and quantitatively determined collagen type I expression.

The statistical analysis was performed with SPSS software (SPSS Inc, Chicago, USA) version 16.0 using methods of descriptive statistics, Mann-Whitney test, Kruskal-Wallis test, independent t test and one-way ANOVA with post hoc Tukey's HSD test. Spearman's and Pearson's correlation coefficients were applied for correlation analyses.

Results

This study used the samples obtained during total knee arthroplasty performed in 30 women and 5 men. Initially, the samples from 39 consecutive patients were collected, but the samples of four patients were excluded because three patients used chondroprotective drugs and one had infectious arthritis. All patients were operated with the diagnosis of idiopathic arthrosis. This is the first operative intervention on the knee in all patients except one, who had undergone synovectomy before total knee arthroplasty. In all patients, the anatomic graduated component (AGC) monoblock knee endoprosthesis was implanted. The youngest patient was 56 and the oldest was 78 years old (the average age being 69.31 years). The average BMI of our patients was 30.8, 60% of the patients were obese, 28.57% were overweight and 11.43% were physiologically nourished.

Anterior cruciate ligament (ACL) was preoperatively intact in most patients (60%), and preoperative angulation of knee joint was predominantly varus (80%). Further on, regarding the preoperative contracture of knee joint, there was a flexion contracture in more than half of patients (65.71%), the mixed flexion/extension contracture was found in 22.86% of patients, while 5.71% of patients had extension contracture and another 5.71% of patients had no contracture at all. Due to the small number of patients without contractures and with extension contracture, the expression of collagen type I in these patients could not be statistically compared with the patients who had other two types of contractures.

Seventy obtained menisci were classified using macroscopic classification according to Sun et al. [19]. Most of them had grade 4 of damage (30%), 27.14% had grade 3, while the other degrees were significantly less present: grade 2, grade 1 and 0 were found in 15.71%, 14.29% and 12.86% of the menisci, respectively. In all analyzed menisci, varus angulation led to larger damage to the medial meniscus, while lateral menisci were more damaged in valgus angulation.

The control group was formed based on these results (10 menisci- 9 lateral and 1 medial). The samples from this group were used to compare the structural changes in the degenerated menisci. Out of 35 analyzed menisci in the experimental group, 27 were medial (77.14%) and 8 were lateral (22.86%) menisci; 45.71% of samples had grade 3, and a slightly larger number of samples (54.29%) had grade 4.

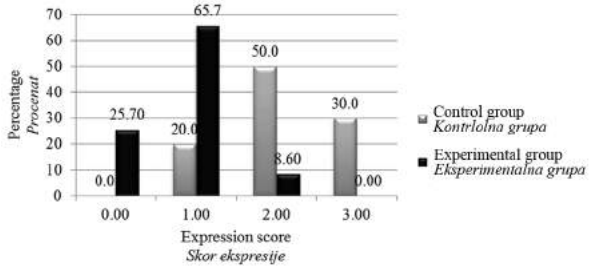
The semiquantitative scale was used for the first estimation of semiquantitative expression of collagen type I. The percentages of medians of four expression scores show the preservation of this type of collagen in the control samples and a decreased expression in the samples from the experimental group **(Graph 1)**.

In the control samples, the expression of collagen type I was highest in the body of meniscus, whereas it was lower in both horns. In the experimental group, there was a significant decrease in the expression (score 1 in all three parts of the meniscus). The comparison of the semiquantitatively determined expression of collagen type I between the control and experimental group showed that the

Table 1. Expression of collagen type I on semiquantitative scale*
Tabela 1. *Ekspresija kolagena tipa I na semikvantitativnoj skali**

	Control group (median) *Kontrolna grupa (medijana)*	Experimental group (median) *Eksperimentalna grupa (medijana)*	U	Z	p	Effect size (r) *Veličina efekta (r)*
Anterior horn *Prednji rog*	2	1	71	-3.097	0.002†	0.46‡
Body/*Telo*	3	1	11	-4.724	0.000†	0.7§
Posterior horn *Zadnji rog*	2	1	35	-4.182	0.000†	0.62§

* Mann-Whitney U test; † statistically significant difference; ‡ medium effect size; § large effect size
* *Man-Vitnijev U-test; † statistički značajna razlika; ‡ umerena veličina efekta; § jaka veličina efekta*



Graph 1. Percentage ratio of semiquantitatively determined expression of collagen type I in both study groups
Grafikon 1. *Procentualni odnos semikvantitativno određene ekspresije kolagena tipa I u obe ispitivane grupe*

expression of collagen type I differed statistically significantly between these two groups, the largest decrease in the expression being in the body of meniscus **(Table 1)**.

There were no statistically significant differences in the expression of collagen type I between men and women (U = 58, Z = -0.958, p = 0.338). When correlating the expression of collagen type I with the age and BMI, a slight decrease in the expression of this type of collagen was observed in relation to the age (ρ = -0.001, p = 0.994). In addition, the obese patients had a lower expression (ρ = -0.296, p = 0.085). However, the correlation was weak in both cases.

Among various forms of preoperative contractures of the knee joint there was no difference in the expression of collagen type I (the difference between flexion and flexion/ extension contracture: U = 91, Z = -0.054, p = 0.957, r = 0.009). No difference was detected between the groups of patients with the intact and absent anterior cruciate ligament and it was p= 0.146 for the anterior horn, p= 0.611 for the body and p= 0.669 for the posterior horn. There was no significant difference in the expression of collagen type I between varus and valgus angulation of the knee joint (for the anterior horn p = 0.467, for the body p = 0.289 and for the posterior horn p = 0.06).

When the presence of a certain angulation of the knee joint and the integrity of the ACL in those angulations were taken together and compared with the semiquantitative expression of collagen type I, no statistically significant difference was found in the expression in these 4 groups of our study samples (Kruskal-Wallis's test, λ^2 (3, n = 35) = 3.359, p = 0.339).

The software quantification of staining intensity score of collagen type I did not show a statistically significant difference among the samples of control and experimental group **(Table 2)**.

More intense staining of collagen type I was observed in the posterior meniscal horns in the female subjects ($\bar{x}$=23.663, SD=17.874) than in the male patients ($\bar{x}$=5.345, SD=2.541), the difference being statistically significant (t (32. 947) = -5.301, p = 0.000, eta squared = 0.459). More intense staining of this part of the meniscus was noted in the patients with higher BMI (r = 0.385, p = 0.022). No difference between the genders and the correlation with BMI, which was seen in the posterior horn, was found in the body and the anterior horn of the meniscus.

Table 2. Staining intensity scores of collagen type I in relation to study groups and anatomical part of meniscus*
Tabela 2. *Skorovi intenziteta bojenja kolagena tipa I u odnosu na ispitivane grupe i anatomski deo meniskusa **

	Control group *Kontrolna grupa ($\bar{x}$ ± SD)*	Experimental group/*Eksperimentalna grupa ($\bar{x}$ ± SD)*	t	p	95% Confidence interval/*Razlika aritmetičkih sredina (95% CI)*	Eta Squared *Eta kvadrat*
Anterior horn *Prednji rog*	19.278 ± 6.003	20.219 ± 14.736	-0.3 (df=37.354)	0.766	-0.94 (-10.62 to 8.74)	0.002
Body/*Telo*	20.364 ± 10.043	19.983 ± 15.085	0.075 (df=43)	0.941	0.381 (-9.872 to 10.634)	0.0001
Posterior horn *Zadnji rog*	18.608 ± 14.81	21.047 ± 17.763	-0.396 (df=43)	0.694	-2.438 (-14.867 to 9.99)	0.003

*independent samples t-test/**nezavisni t-test*

Table 3. Differences of staining intensity scores of collagen type I in relation to anatomical part of meniscus and three examined characteristics of patients and joint changes*†
Tabela 3. *Razlike skorova intenziteta bojenja kolagena tipa I u odnosu na anatomski deo meniskusa i tri ispitivane karakteristike pacijenata i zglobnih promena *†*

Staining intensity score of collagen type I and: *Skor intenziteta bojenja kolagena tipa I i:*	Anterior horn *Prednji rog*	Body *Telo*	Posterior horn *Zadnji rog*
1. preoperative contracture* *1. preoperativne kontrakture**	t(29)= 0.274, p=0.786	t(29)= - 0.469, p=0.643	t(29)= 0.296, p=0.769
2. age of patients† *2. doba pacijenata†*	r=0.004, p=0.980	r=0.148, p=0.396	r=-0.119, p=0.494
3. condition of anterior cruciate ligament* *3. stanja prednje ukrštene veze**	t(33)= - 1.599, p=0.119	t(33)= - 0.983, p=0.333	t(33)= - 0.853, p=0.4

*independent samples t-test; † Pearson's correlation/**nezavisni t test; † Pirsonov test korelacije*

As for the preoperative angulation, the quantitative analysis showed a greater preservation of collagen type I in the anterior horn of valgus knees compared to varus (t (33) = - 2.935, p = 0.006, eta squared = 0.2). These differences were not seen in other parts of the meniscus.

More intense staining of collagen type I in one part of the meniscus was not related to more intense staining in another part (the correlation of expression being the anterior horn - body: r = 0.194, p = 0.263; the body- posterior horn: r = 0.133, p = 0.446; the anterior horn- posterior horn: r = 0.102, p = 0.558). The presence of preoperative contracture and integrity of ACL did not affect the staining intensity score of collagen type I. Neither was there an apparent correlation between the age and this score **(Table 3)**.

Regarding the surface stained on collagen type I, the largest area in both studied groups was in the posterior meniscal horns. The comparison of samples between the control and experimental group showed a reduction of stained surface in the meniscal body, which was not observed in the meniscal horns. However, this decrease was not statistically significant **(Table 4)**.

Similar to the staining intensity score, a statistically significant difference in the surface stained on collagen type I was observed only in the posterior meniscal horns of male and female patients. The menisci of women had a larger stained surface for this type of collagen($\bar{x}$=157.61, SD=121.151) than of the men ($\bar{x}$=39.913, SD=18.84), and this difference was highly statistically significant (t (32.991) = -2.142, p = 0.000, eta squared = 0.122). In addition, a higher BMI meant a larger stained surface of this part of the meniscus (Pearson's correlation, r = 0.419, p = 0.012). The comparisons of staining surface in other parts of the meniscus with the gender and BMI revealed no statistically significant differences.

A larger surface stained on collagen type I in one part of the meniscus did not mean a larger stained surface in another part (the correlation of expression being the anterior horn - body: r = 0.101, p = 0.565; the body-posterior horn: r = 0.138, p = 0.429; the anterior horn - posterior horn: r = 0.05, p = 0.777). Different forms of preoperative contracture, condition of ACL, preoperative angulation and age of the patient did not lead to differences in the surface stained on collagen type I in different parts of the meniscus **(Table 5)**.

The comparison of the grade of macroscopic damage and the semiquantitatively determined expression of collagen type I showed that the damaged menisci had a lower expression of this type of colla-

Table 4. Differences in surface of visual field (in square micrometers) stained for collagen type I in relation to study groups and anatomical part of meniscus*
Tabela 4. *Razlike površina vidnog polja (u μm2) obojenih na kolagen tipa I u odnosu na ispitivane grupe i anatomski deo meniskusa**

	Control group ($\bar{x}$ ± SD) *Kontrolna grupa ($\bar{x}$ ± SD)*	Experimental group ($\bar{x}$ ± SD) *Eksperimentalna grupa ($\bar{x}$ ± SD)*	t (43)	p	95 % Confidence interval Razlika aritmetičkih sredina (95 % CI)	Eta Squared Eta kvadrat
Anterior horn *Prednji rog*	132.71 ± 64.57	136.97 ± 110.94	-0.115	0.909	-4.253 (-78.72 do 70.21)	0.0003
Body *Telo*	140.32 ± 62.02	132.33 ± 105.44	0.227	0.821	7.983 (-62.85 do 78.817)	0.0011
Posterior horn *Zadnji rog*	140.93 ± 120.57	140.796 ± 119.612	0.03	0.998	0.135 (-86.05 do 86.77)	0.00002

*independent samples t-test/**nezavisni t-test*

Table 5. Relationship among surface stained for collagen type I and four examined characteristics of patients*†
Tabela 5. *Poređenje površine obojene na kolagen tipa I sa četiri karakteristike pacijenata*†*

Surface stained on collagen type I and: *Površina obojena na kolagen tipa I i:*	Anterior horn *Prednji rog*	Body *Telo*	Posterior horn *Zadnji rog*
1. preoperative* contracture 1. *preoperativne* kontrakture*	t(29)= 0.398, p=0.694	t(29)= -0.408, p=0.687	t(29)= 0.119, p=0.906
2. age of patients† 2. *doba pacijenata†*	r= -0.104, p=0.551	r= 0.200, p=0.249	r= -0.146, p=0.403
3. condition of anterior cruciate ligament* 3. *stanja prednje ukrštene veze**	t(33)= -1.891 p=0.067	t(18.906)= -1.395, p=0.179	t(33)= 0.924, p=0.366
4. preoperative axis* 4. *preoperativne osovine**	t(33)= -1.893, p=0.067	t(33)= 0.909, p=0.37	t(33)= 0.507, p=0.616

*independent samples t-test; † Pearson's correlation/*nezavisni t test; † Pirsonov test korelacije

gen. Kruskal-Wallis's test showed a highly statistically significant difference among three studied grades of macroscopic damage (grade 1,3 and 4) in all three parts of the meniscus, as follows: anterior horn- λ^2 (2) = 13.692, p = 0.001; body λ^2 (2) = 20.868, p = 0.000; posterior horn- λ^2 (2) = 15.721, p = 0.000. The comparison of macroscopic damage with staining intensity score and stained surface with one-way ANOVA showed that among different grades of macroscopic damage the difference in these parameters was found only in the anterior horn (for staining intensity score p = 0.03, for stained surface p= 0.025). In order to determine which grades of macroscopic damage were the source of these differences, we performed post-hoc analysis Tukey's HSD test, which showed that the source was different between the macroscopic grades 3 and 4 in the staining intensity score (p = 0.014) and stained surface (p = 0.024).

Discussion

Menisci of the knee joint are part of a complex system that should ensure the stability and weight carrying the body during the movements of the joint. In addition, the medial and lateral menisci are an integral component of normal articular homeostasis. When some pathological changes occur in the meniscus, such as acute clefts or chronic degeneration, the whole cascade of processes activates and causes changes of biomechanical compositions, ultrastructural organization, macroanatomical shape and molecular organization. It is very important to understand these changes when analyzing the progression of the disease in meniscus, which could eventually lead to osteoarthritis.

The antibody against collagen type I, which was used in this study, does not react with thermally denatured collagen, so the results shown here are related only to collagen type I which was present unaltered in the analyzed samples. The results of semiquantitative analysis showed that there was a decrease in the expression of collagen type I in the experimental, OA menisci, in comparison to the control group. Mine et al. examined whether there were differences between the menisci in the knees which did not show degenerative changes and those which, due to advanced degenerative changes, required total knee arthroplasty. In the menisci without degeneration, the expression of collagen type I varied among the patients and it was evident throughout the entire meniscus (except for peripheral vascular area). An intensive expression existed in both circumferential and radial fibers. These authors described a decrease in the expression of collagen type I in the degenerated menisci, and this type of collagen was totally absent in some cases of very severe degeneration [17]. Sun et al. [16] observed a higher content of collagen in the normal menisci compared to the OA menisci. The loss of collagen in the OA menisci was expressed in all areas of the meniscus, more so in the medium and deeper zones than at the surface. In addition, there was a statistically significant reduction of collagen type I expression in OA menisci in their samples. Using the method of collagen densitometry by converting the image to the average levels of gray, Mine et al. got the mean expression of collagen type I of 20.13 in the degenerated menisci, which was less than in the menisci without degeneration, where this value was 151.08. Such a big difference was not observed for type II collagen [17]. These authors believe that the limit of reparation was exceeded in their samples, which led to such a large decrease in the density of collagen type I and loss of tissue structure. The reduction of expression of collagen, observed in our study and cited literature, illustrates the contrast between the meniscus and articular cartilage of the knee joint during OA, because the expression of these two types of collagen increases with the severity of degeneration in the articular cartilage [20].

A decrease in the expression of collagen type I in the semiquantitative analysis was most observed in the body of meniscus, while such a loss was less in the meniscal horns. The intactness of the horns of the meniscus, in particular of the anterior one, was observed when correlating the macroscopic damage with the expression of collagen type I. According to our results, a higher grade of macroscopic damage is associated with a lower expression of collagen type I in the meniscus, wherein the level

of such negative correlation was high for the body and the posterior horn and moderate for the anterior horn. In our samples, the anterior horn was more preserved in valgus knees. Literature data show that the changes are more noticeable in the body and the posterior horn in the OA menisci, while the anterior horn remains well preserved, with only a few signs of matrix loss or degeneration [21]. This was also confirmed by Pauli et al. [22], who concluded that the anterior horns of both menisci were less affected, macroscopically and microscopically. However, an increase in gene expression of procollagen I was observed in these horns, which is considered to be a result of reparative response that occurs in the OA menisci [21]. During the healing of fibrous tissue, such as ligaments and tendons, the fibroblasts exhibit an increased expression of type I procollagen [23], so it is assumed that the same mechanism exists in the tissue of the meniscus.

In order to analyze the expression of collagen type I more precisely, the quantitative analysis was performed in addition to the semiquantitative analysis. The previous ways of quantifying the expression of certain substances did not take into account the possibility that the substance may be present in varying concentrations. To overcome this problem, an algorithm has been developed, which assigns different color scores to different shades of a particular color representing positive staining, so that precise values are assigned to certain shades of color. Therefore, we decided to perform the quantification using software TissueQuant. This form of quantifying the expression of collagen type I did not show statistically significant differences between the control and experimental group. In the control group, the highest value of staining intensity score was seen in the meniscal body, and the lowest one in the posterior horn. The samples in the experimental group differed because the highest expression was observed in the horns, some more in the posterior one. Statistically significant quantitative differences in the expression of collagen type I were observed only in the menisci of the experimental group, and only in the posterior horn. Higher staining intensity scores, as well as the size of stained surface in this horn, were observed in women, and there was a positive correlation between these scores and BMI. These associations were not found in the body and the anterior horn of the meniscus. On the basis of NMR images in T2 sequences, Chiang et al. [24] found that the collagen content in the posterior horn of meniscus in asymptomatic volunteers from all age groups was higher in women and increased with the age of female subjects.

Although the staining intensity score was the same in both groups, certain differences were observed on the surface stained for collagen type I. A decrease in the stained surface was seen in the body of meniscus from 140.32 μm^2 in control to 132.33 μm^2 in the experimental group; however, it was not statistically significant. This reduction was not observed in the horns. The results of quantitative studies of Katsuragawa et al. [21] showed that in the OA menisci there was a significant reduction in the average diameter of collagen fibrils and the percentage of area where these fibrils were placed, but at the same time there was an increase in the number of fibrils per unit of area. Wen et al. [25] found that collagen fibers became thicker in the beginning and during the progression of OA, which could explain the decrease in the area stained for collagen type I. Sun et al. [16] observed that the collagen network of OA meniscus was less organized and less compact than the normal menisci, that being in accordance with the differences found between our two study groups.

Conclusion

In the semiquantitative evaluation of osteoarthritic menisci, the expression of collagen type I marked by immunohistochemical staining of anti-collagen I antibody was significantly lower compared to the macroscopically unaltered menisci. A decrease in the level of expression correlates with an increase in the grade of macroscopic damage of the meniscus. A decrease in the expression is larger in the body of the meniscus than in the horns. In the quantitative evaluation of expression of collagen type I there was a mild, statistically non-significant decrease in the stained surface in the experimental group compared with the control group, but only in the body of the meniscus, while there were no differences in the horns. No changes were found in the immunohistochemical staining intensity score between the two study groups. A more intense expression of collagen type I was seen in the posterior horn of the meniscus of women than of men. There was also a positive correlation of quantitatively estimated expression with body mass index. The expression of collagen type I in the anterior horn was significantly lower in varus than in valgus knees.

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ALGORITHM OF OVULATION INDUCTION IN PATIENTS WITH POLYCISTIC OVARY SYNDROME

ALGORITAM INDUKCIJE OVULACIJE KOD PACIJENTKINJA SA SINDROMOM POLICISTIČNIH JAJNIKA

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Summary

Introduction. Polycystic ovary syndrome is the most frequent endocrine disturbance in the reproductive period of women's life and the most frequent cause of anovulatory infertility. Ovulation and pregnancy in patients having polycystic ovary syndrome may be a result of a wide range of therapeutic options, and the treatment assumes a gradual approach – from simple noninvasive to expensive and demanding procedures. **Material and Methods**. A systematic literature survey concerning the efficiency of particular ovulation induction methods in respect of the reproductive outcome was carried out with the aim of establishing the algorithm for ovulation induction in infertile patients having polycystic ovary syndrome. The search was confined to clinical investigations performed on human subjects, reported in English in the period from the beginning of 2010 to June of 2014. **Conclusion.** As a conclusion of this systematic survey of the efficiency of ovulation induction methods, which confirms and supplements the knowledge in this field, it is possible to form the algorithm for ovulation induction in infertile patients having polycystic ovary syndrome, consisting of the following subsequent steps: 1) modification of life style, 2) induction with clomiphene citrate 3) use of metformin, 4) use of aromatase inhibitors, 5) application of gonadotropins and laparoscopic ovarian drilling – as a second-line treatment, and 6) assisted reproductive techniques.

Key words: Ovulation Induction; Polycystic Ovary Syndrome; Algorithms; Treatment Outcome; Anovulation; Infertility, Female; Life Style; Clomiphene; Metformin; Aromatase Inhibitors; Gonadotropins; Laparoscopy; Reproductive Techniques, Assisted

Sažetak

Uvod. Sindrom policističnih jajnika predstavlja najčešći endokrini poremećaj u reproduktivnom periodu žena, najčešći uzrok anovulatornog infertiliteta. Ostvarivanje ovulacije i trudnoće kod ovih pacijentkinja može biti rezultat primene širokog spektra terapijskih opcija, tako da tretman predviđa postepen pristup – od jednostavnijih, neinvazivnih do skupih i zahtevnih procedura. **Materijal i metode.** Sproveden je sistematičan pregled literature o efikasnosti pojedinih metoda indukcije ovulacije u odnosu na reproduktivni ishod radi utvrđivanja algoritma indukcije ovulacije za lečenje infertiliteta kod pacijentkinja sa sindromom policističnih jajnika. Pretraga je ograničena na klinička istraživanja sprovedena isključivo na ljudskim subjektima, objavljena na engleskom jeziku u periodu od početka 2010. godine do juna 2014. godine. **Zaključak.** Kao zaključak ovog sistematičnog pregleda o efikasnosti metoda za indukciju ovulacije, koji potvrđuje i dopunjuje prethodna saznanja iz ove oblasti, može se formirati terapijski algoritam za indukciju ovulacije kod pacijentkinja sa sindromom policističnih jajnika, koji čine sledeći koraci: 1) modifikacija životnog stila; 2) indukcija ovulacije klomifen-citratom; 3) primena metformina; 4) upotreba inhibitora aromataze; 5) primena gonadotropina i laparoskopska punkcija jajnika – kao druga terapijska linija i 6) asistirane reproduktivne tehnike.

Ključne reči: Indukcija ovulacije; Sindrom policističnih jajnika; Algoritmi; Ishod lečenja; Anovulatorni ciklus; Infertilitet žene; Životni stil; Klomifen; Metformin; Inhibitori aromataze; Gonadotropini; Laparoskopija; Asistirane reproduktivne tehnike

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in the women's reproductive period, the most frequent cause of hyperandrogenism, oligoanvulation and the anovulatory infertility [1, 2]. Metabolic disorders (obesity, insulin resistance, hyperinsulinemia) are often associated with this disorder, but it is not yet clear if they are part of the disease or comorbidities [3]. PCOS amounts to 16.6% according to the results of the most recently published study, which is at the same time the most comprehensive study of PCOS prevalence based on the widely accepted criteria of the Rotter-

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Abbreviations
PCOS – polycystic ovary sindrome
BMI – body mass index

dam consensus 2003. The therapeutic approaches differ in dependence of whether the reproductive problems are of primary concern, since PCOS is the most frequent cause of anovulatory infertility, or there are some other PCOS consequences. In view of the fact that ovulation and pregnancy in patients with PCOS can be a result of some less aggressive and more economic therapeutic methods, the treatment assumes a gradual approach – from simple noninvasive to expensive and demanding procedures.

The therapeutic modalities for ovulation induction mentioned in the literature are: body mass reduction, use of metformin, clomiphene citrate, aromataze inhibitors, gonadotropins, and surgical techniques of ovulation induction – wedge resection of ovaries, which is abandoned in modern times, and laparoscopic ovarian drilling [4–9].

Starting from the data obtained by the systematic survey of the literature concerning particular ovulation induction methods in respect of the preproductive outcome, the aim of this paper was to establish an algorithm for the induction of ovulation, to treat the infertility in patients with PCOS.

Material and Methods

A detailed search of the literature was carried out in September of 2014 using the PubMed and Scopus electronic databases. The keywords were: *'lifestyle modification' OR 'clomiphene citrate' or 'letrozole' or 'metformin' or 'gonadotrophins' or 'IVF' and 'PCOS' and 'induction ovulation' and 'ovulation' and 'pregnancy'*. The search was limited to clinical investigations carried out on human subjects, published in English in the period from the beginning of 2010 to June 2014.

The search was made of the corresponding abstracts with the aim of identifying the papers relevant to the subject of the study. Full texts of all the pertinent studies accessible via the Academic Network of Serbia and via the Serbian Library Consortium for Coordinated Acquisition (KoBSON) were collected and analyzed.

The criteria for inclusion into the systematic literature search were: 1) study of the type of a randomized controlled study and 2) reproductive outcome (rate of ovulation or pregnancy) as a measure of the effect of a given therapeutic procedure. The selection excluded the studies concerning systematic surveys and/or meta-analyses, as well as review articles.

Data extraction – From each selected study the following data were extracted: author(s) name(s), number of subjects included in the study, interventions performed, estimated outcome, results, and conclusion.

Results

Out of 72 studies identified in this research, 54 were found via the PubMed and 18 via the Scopus database. One study was excluded as a duplicate, and 27 studies were concluded not to be related to the research subject. Since the full text was not available for 12 studies, they also had to be excluded. After searching the abstracts of the remaining studies, 7 were excluded because of the lack of data about the outcome of interest, and 14 were the articles of a systematic review type, meta-analyses, and review articles. Finally, 11 studies remained to be included in this survey, and they were meticulously studied to draw the required data.

The samples of all studies included women with PCOS and chronic anovulation. The estimated outcomes of interest were the ovulation rate (OR) and/or pregnancy rate (PR), and, where it was available, the live birth rate was also taken into account. The results and conclusions of the studies are given in **Table 1.**

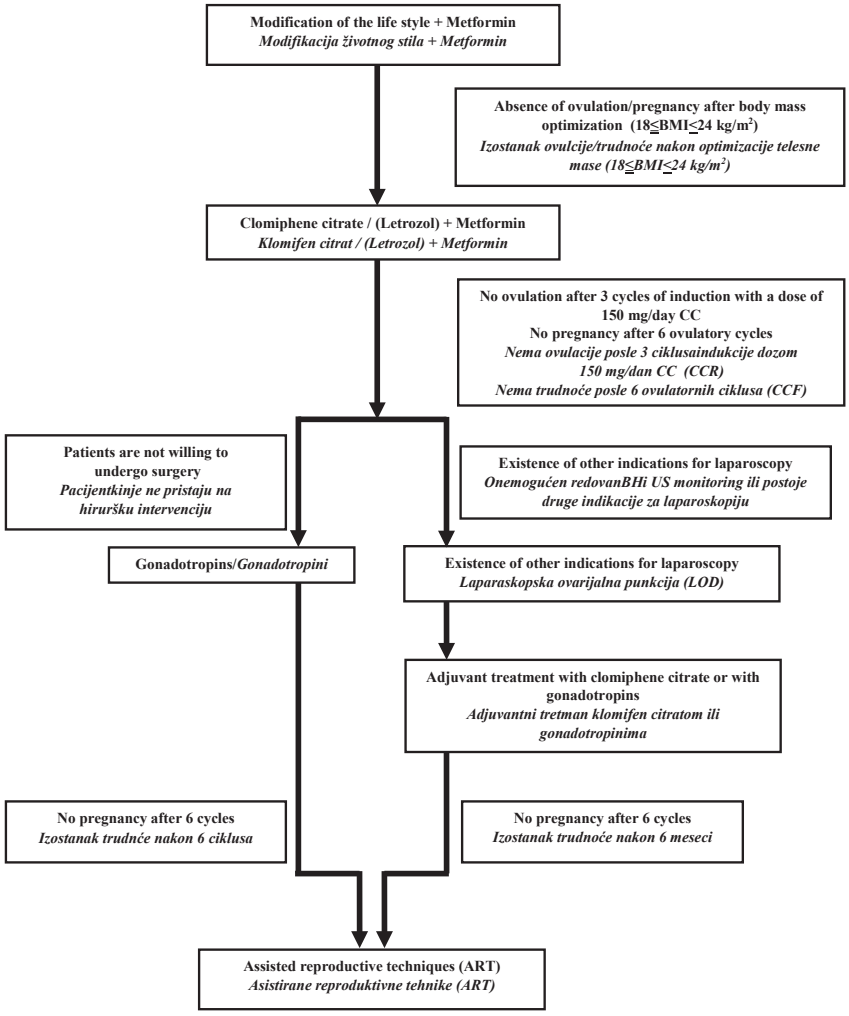
Discussion

The body mass index (BMI) is known to be in opposite correlation with the response to some drugs that are used in the ovulation induction, and the modification of life style and decrease in the body weight improve the response to the ovulation inductors and contribute to a better reproductive outcome [10–12]. Such an attitude was also confirmed by the results of a study encompassed by this survey [13]. The study of Morin-Papunen et al. showed the statistical significance of the correlation between a three-month pretreatment with metformin and better reproductive outcome, which was especially pronounced in obese patients [14]. Having compared the efficiency of clomiphene citrate and metformin, Baran et al. arrived at the conclusion that ovulation was more frequent in those patients who received clomiphene citrate, although the difference was not statistically significant. Therefore, they have recommended clomiphene citrate as the first line treatment [15]. A similar conclusion was also reached by Homburg et al. [16]. Although the results regarding the reproductive outcome are in favor of the application of gonadotropins, taking into account the overall costs and treatment conformity, the conclusion of this study is that clomiphene citrate should still be considered as the first choice treatment. If ovulation failed to occur after three cycles of induction with clomiphene citrate at a dose of 150 mg/day, such patients are considered to be resistant to clomiphene citrate. Abu Hashim et al., Begum et al. and Abd Elgafor et al. have concluded that the application of metformin in combination with standard ovulation inductors contributes to a better reproductive outcome [17–19]. This finding verifies the recommendations of a number of literature sources proposing that the next step in clomiphene citrate-resistant patients should be the introduction of metformin in therapy since it results in a significantly improved response to clomiphene citrate. This is especially important in case of the patients with BMI>25 kg/m^2 and insulin resistance [1]. The results of earlier investigations showed that aromatase inhibitors (i.e. letrozole) ap-

Table 1. Efficiency of ovulation induction methods in patients with PCOS: characteristics of the encompassed studies
Tabela 1. *Efikasnost metoda indukcije ovulacije kod pacijentkinja sa PCOS: karakteristike uključenih studija*

Author *Autor*	N	Intervention *Intervencija*	Assessment *Procena*	Results/*Rezultat*	Conclusion/*Zaključak*
Begum et al. (2013)	165	Pretreatment with M 1500 mg/day 4 weeks A: M + CC (150 mg/day, 5 days) *Pretretman M 1500 mg/dan 4 nedelje* *A: M + CC (150 mg/dan, 5 dana)* B: M + rFSH3 (75 IU) C: rFSH (75 IU)	OR PR	OR A: 27.27% B: 89.09% C: 74.55% PR A: 12.73% B: 54.55% C: 29.09%	Metformin enhances the response to the ovulation inductors and is safe for the application in the case of PCOS/*Metformin povećava odgovor na induktore ovulacije i bezbedan je za upotrebu u PCOS*
Abd Elgafor (2013)	146	A: M (1700 mg/day) + L (5 mg/day, 5 days), B: LOD/*A: M (1700 mg/dan) + L (5 mg/dan, 5 dana), B: LOD*	OR PR	NS!	Both treatments (M+L and LOD) were equally efficient as second line treatment in CCR patients/*Oba tretmana (M+L i LOD) su podjednako efikasna kao tretman 2. linije kod CCR pacijentkinja*
Morin Papunen et al. (2012)	320	A: M obese 1500 mg/day; normal body mass – 1000 mg/day) B: placebo *A: M gojazne 1500mg/dan; normalna telesna masa - 1000 mg/dan), B: placebo*	PR LBR	PR A: 53.6% B: 40.6% LBR A: 41.9% B: 28.8%	Metformin improved the reproductive outcome significantly, especially in obese women, when applied as a three-month pretreatment or in combination with routine means for ovulation induction/*Metformin značajno poboljšava reproduktivni ishod posebno kod gojaznih žena primenjen kao tromesečni pretretman ili u kombinaciji sa rutinskim sredstvima za indukciju ovulacije*
Homburg et al. (2012)	302	A: CC (50-150 mg/day, 5 days) B: rFSH (50IU) *A: CC (50-150 mg/dan, 5 dana)* *B: rFSH (50IU)*	PR LBR	PR A: 41.2% B: 52.1% LBR A: 36.9% B: 47.4 %	Because of the comfort and price, it is recommended that CC should be first line treatment, although low doses of FSH showed better results. Low dose FSH may be first line treatment in older patients./*Zbog komfora i cene preporučuje se da CC bude tretman 1. linije, iako niske doze FSH pokazuje bolji rezultat. Niske doze FSH može biti tretman 1. linije kod starijih pacijentkinja.*
Banerjee Ray (2012)	147	A: CC 100 mg/day, 5 days B: L 2.5 mg /day, 5 days *A: CC 100 mg/dan, 5 dana* *B: L 2,5 mg /dan, 5 dana*	OR PR	OR A: 61.5% B: 86.9% PR A: 17.9% B: 28.9%	L is safe and equally efficient as CC when ovulation is concerned. In contrast to CC, L has a positive effect on the endometrium, potentially increasing the probability of pregnancy after the successful induction of ovulation./*L je bezbedan i podjednako efikasan kao i CC u postizanju ovulacije. Za razliku od CC, L ima pozitivan efekat na endometrijum, čime se potencijalno povećava verovatnoća trudnoće nakon uspešne indukcije ovulacije.*
Ramezan Zadeh et al.	67	A: L 5 mg/day B: L 7,5 mg/day *A: L 5 mg/dan* *B: L 7,5 mg/dan*	OR PR	OR A: 90% B: 89.2% PR A: 25.8% B: 21.2% NS !	A 7.5 mg/day dose of L is not advantageous over a 5 mg/day dose of L as first line treatment. A higher dose of L is not advantageous in respect of the thickness of the endometrium. *Doza L od 7,5mg/dan ne predstavlja prednost u odnosu na dozu 5 mg/dan L kao tretmana prve linije. Veća doza letrozola nema prednosti ni u pogledu debljine endometrijuma.*
Abu Hashim et al. (2011)	282	A: M (1500 mg/day) + CC (50-150 mg/day) B: LOD *A: M (1500 mg/dan) +* *B: LOD*	OR PR	OR A: 67.0% B: 68.2% PR A: 15.4% B: 17.0% * all patients with BMI< (24.8+/-1.7) achieved ovulation in the M-CC treatment/** sve pacijentkinje sa manjim BMI (24,8+/-1,7) postigle su ovulaciju M-CC tretmanom*	The M-CC combination is equally efficient in achieving ovulation and pregnancy as LOD in CC-resistant patients during six months. It is recommended that the CC-M treatment should be preferable in younger patients. For patients over 35, the recommendation is the induction with gonadotropins or IVF. All obese patients need to optimize their body mass. *Kombinacija M-CC je podjednako efikasna u postizanju ovulacije i trudnoće kao i LOD kod CC rezistentnih pacijentkinja tokom 6 meseci praćenja. Predlaže se da se tretmanu CC-M da prednost kod mlađih pacijentkinja. Za pacijentkinje iznad 35 god. predlaže se indukcija gondotropinima ili IVF. Sve gojazne pacijentkinje trebale bi da optimizuju telesnu masu.*
Palomba et al. (2010)	96	A: 6-week physical activity + diet (1000 kCal/day) B: CC (150 mg/day) C: physical activity + diet + CC (150 mg/day) *A: 6 nedelja fizičke aktivnosti + dijeta (1000 kCal/dan)* *B: CC (150 mg/dan)* *C: fizička aktivnost + dijeta + CC (150 mg/dan)*	OR	OR – statistically significantly higher ovulation rate in the group C (37.5%) vs. group A (12.5%) and B (9.4%) *OR - statistički značajno viša stopa ovulacije u grupi C (37.5%) u odnosu na grupu A (12.5%) i B (9.4%)*	Six weeks of physical activity + hypocaloric diet increase the probability of ovulation induced with CC in the CCR overweight or obese patients. *6 nedelja fizičke aktivnosti + hipokalorijska dijeta tretmana, povećava verovatnoću ovulacije indukovane CC kod CCR pacijentkinja sa prekomernom telesnom težinom ili gojaznih.*
Abu Hashim et al. (2010)	260	A: L 2,5 mg/day d3-7 B: LOD (6 months) *A: L 2,5 mg/dan d3-7* *B: LOD (6 meseci praćenja)*	OR PR LBR	OR: NS A: 65.4% B: 69.3% PR: A: 15.6% B: 17.5%	L and LOD are equally efficient in the induction of ovulation and achievement of pregnancy in CCR patients, but in view of the lower invasiveness and treatment costs, L should be advantageous over LOD./*L i LOD su podjednako efikasni u indukciji ovulacije i postizanju trudnoće kod CCR pacijentkinja, ali obzirom na manju invazivnost i cenu tretmana, L bi trebalo da ima prednost nad LOD.*
Baran et al. (2010)	69	A: M1700 mg/day B: CC 50-150 mg/day *A: M1700 mg/dan* *B: CC 50-150 mg/dan*	OR PR	OR: A:32.3 % B: 60.6% PR: NS A:36.6 % B:35.4%,	CC and M as first line drugs enhanced the pregnancy rate; CC gave better results in achieving ovulation./*CC i M kao medikamenti 1. linije povećavaju stopu trudnoća. CC daje bolje rezulatate u postizanju ovulacije.*
Nejad et al. (2011)	366	IVF	PR	PR: NS	The outcomes of IVF with PCOS patients and patients with tubal infertility are similar./*Ishod IVF kod PCOS pacijentkinja i pacijentkinja sa tubarnim infertilitetom je sličan.*

Legend: M – metformin, CC – clomiphene citrate, rFSH – recombinant follicle-stimulating hormone, L – letrozole, LOD – laparoscopic ovarian drilling, IVF – *in vitro* fertilization, NS – not statistically significant, OR – ovulation rate, PR – pregnancy rate, LBR – live birth rate
Legenda: M= metformin, CC= klomifen citrat, rFSH= rekombinantni folikulo-stimulirajući hormon, L= letrozol, LOD= laparoskopska punkcija jajnika, IVF= in vitro fertilizacija, NS= nije statistički značajno, OR – stopa ovulacije, PR – stopa trudnoće, LBR – stopa živorođene dece, PCOS - sindrom policitičnih jajnika



Scheme 1. Treatment algorithm for the ovulation induction in PCOS patients
Schema 1. *Terapijski algoritam indukcije ovulacije kod pacijentkinja sa sindromom policističnih jajnika*

peared to be another candidate for the first choice drug [20]. A main advantage of letrozole over clomiphene citrate is its positive effect on the endometrium. This was confirmed by the study of Banerjee Ray et al. [21]. Ramenzanzadeh et al. found that there was no significant difference in the reproductive outcome when higher daily doses of letrozole were administered [22]. In view of the advantages of letrozole over clomiphene citrate, this drug could be also an adequate option for second choice treatment in clomiphene citrate-resistant patients. Two studies from this survey confirmed that letrozole, that is the combination of letrozole and metfomin, were as efficient as laparoscopic ovarian drilling [17, 19]. This survey did not identify any study which had investigated potential teratogenicity of letrozole, and this is, according to some earlier results, one of the main reasons that can limit the use of letrozole in the treatment of ovulatory infertility.

When the reproductive outcome is concerned, the advantage of the application of gonadotropins over clomiphene citrate was also confirmed by the results of this literature search. Begum et al. found that the percentage of ovulatory cycles and achieved pregnancies were statistically significantly higher in the treatments with gonadotropins compared to the combination of clomiphene citrate + metformin [18]. The same study showed that the addition of metformin improved the effect of recombinate follicle-stimulating hormone. A similar result has also been obtained by Homburg et al. pointing out that the high price of gonadotropins is the main reason why they are not a reserve treatment option, that is the first option in older patients [16].

Laparascopic ovarian drilling is another option to be taken into account in case of clomiphene citrate-resistant patients. The studies encompassed by this survey show that there is no statistically significant difference in the rate of ovulation/pregnancy when laparoscopic ovarian drilling is compared to medicamentous treatments – clomiphene citrate + metformin, letrozole alone or letrozole + metformin [17, 19, 23]. Since this is still an invasive

procedure with potential postoperative complications, the recommendation of the studies encompassed by this survey and of some other literature sources is that laparoscopic ovarian drilling should be considered as a second treatment line in the patients that have also some other indications for laparoscopy [9, 17].

If none of the above methods gives desired results, that is when the infertility causes persist, some of the assisted reproductive techniques remain as the last treatment option. Nejad et al. showed that the reproductive outcome of *in vitro* fertilization in PCOS patients is not statistically significantly different compared to that observed in patients with tubal infertility [24], which is also in line with the results of some other studies [25] and meta-analysis [26].

Conclusion

Based on the conclusion that can be drawn from this systematic survey of the methods concerning ovulation induction, which confirms and supplements the previous knowledge in the field, it is possible to form an algorithm for induction of ovulation in patients with polycystic ovary syndrome, which consists of the following steps: 1) modification of the life style accompanied by potential adjuvant therapy with metformin, aimed at optimizing the body mass and solving the problem of insulin resistance, should be the first treatment step for women with body mass index >kg/m^2, which can directly lead to ovulation or contribute to a better effect of the procedures applied in the further treatment; 2) induction of ovulation with clomiphene citrate as a standard first pharmacological line for patients having polycystic ovary syndrome ; 3) application of metformin in combination with clomiphene citrate – which increases the chances of ovulation and gestation so that this combination can be applied from the beginning of the treatment or in case of clomiphene citrate-resistant patients; 4) application of aromatase inhibitors which can substitute clomiphene citrate as the first line treatment, but the proofs of the superiority of letrozole over clomiphene citrate are still insufficiently strong, whereas its application is additionally compromised by its potential teratogenicity; 5) application of gonadotropins and laparoscopic ovarian drilling, which represent the second treatment line; 6. assisted reproductive techniques is the last treatment option if pregnancy does not occur even after six cycles of induction with gonadotropins, that is after six months from the laparoscopic ovarian drilling, or when there are some associated causes of infertility.

The algorithm for induction of ovulation in patients with polycystic ovary syndrome is illustrated in **Scheme 1.**

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IMPORTANCE OF INTEGRATED MANAGEMENT SYSTEM APPLIED IN HEALTH ESTABLISHMENTS IN ORDER TO RAISE TREATMENT QUALITY

ZNAČAJ INTEGRISANOG SISTEMA MENADŽMENTA U ZDRAVSTVENIM USTANOVAMA SA CILJEM PODIZANJA KVALITETA LEČENJA

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Summary

Introduction. The term "management" is best characterized as "managing" economic or social processes to achieve objectives through a rational use of material and immaterial resources by applying the principles, functions, and management methods. This study has been aimed at evaluating the value of an integrated quality management system implemented at the Institute of Cardiovascular Diseases of Vojvodina to improve the quality of treatment. **Material and Methods.** In the period from 2008 to 2010 about 40 employees from the Institute of Cardiovascular Diseases of Vojvodina attended various courses given by the lecturers of the Faculty of Technical Sciences, where the function and significance of the "International Standards Organization" were explained, after which standards of interest were implemented at the Institute of Cardiovascular Diseases of Vojvodina. **Results**. The Department of Cardiology has introduced 11 cardiac procedures with 5 special instructions, 14 general procedures, and 7 specific procedures with 2 instructions. The Department of Cardiac Surgery has introduced 7 procedures to be implemented. The "Vojvodina score" model was put into practice for the perioperative evaluation of cardiac surgery risk. During 2014, the Institute of Cardiovascular Diseases of Vojvodina obtained accreditation for the period of 7 years. **Conclusion.** The integrated quality management system must be applied in order to achieve a high level of health care in the shortest possible time and with the least possible consumption of material and human resources. The application of this system in practice gives a realistic insight into the working processes and facilitates their functioning. It demands and requires constant monitoring of the system efficiency along with continuous changes and improvements of all elements of the working processes and functional units.

Key words: Quality of Health Care; Integrated Advances Information Management System; Quality Indicators, Health Care; Organization and Administration; Health Services

Sažetak

Uvod. Termin *menadžment* najbolje se može okarakterisati kao *upravljanje* privrednim ili društvenim procesima u svrhu postizanja određenih ciljeva putem racionalnog korišćenja materijalnih i nematerijalnih resursa uz primenu principa, funkcija i metoda menadžmenta. Istraživanje je urađeno sa ciljem evaluacije vrednosti integrisanog sistema menadžmenta kvalitetom implementiranog u procedure primenjivane na Institutu za kardiovaskularne bolesti Vojvodine a za podizanje kvaliteta lečenja. **Materijal i metode.** U periodu od 2008. do 2010. godine, oko 40 zaposlenih sa Instituta za kardiovaskularne bolesti Vojvodine prisustvovalo je različitim kursevima koje su realizovali predavači sa Fakulteta tehničkih nauka na kojima su objašnjavani funkcija i značaj Internacionalne/Međunarodne organizacije za standarde (*International Standards Organization*), nakon čega su standardi značajni za našu instituciju implementirani u različite procedure i uputstva. **Rezultati.** Na Klinici za kardiologiju uvedeno je 11 kardioloških procedura sa pet specijalnih uputstava, 14 opštih procedura i sedam specifičnih procedura sa dva uputstva. Na Klinici za kardiohirurgiju implementirano je sedam procedura. Za perioperativnu procenu kardiohirurškog rizika uveden je u praksu *Vojvodina skor* model. Tokom 2014. godine Institut za kardiovaskularne bolesti-Vojvodine dobio je akreditaciju na period od 7 godina. **Zaključak.** Radi postizanja visokog nivoa zdravstvene zaštite u najkraćem mogućem vremenu i uz najmanji mogući utrošak materijalnih i ljudskih resursa, neophodno je implementirati integrisani sistem menadžmenta kvalitetom. Primena ovog sistema u praksi omogućava realan uvid u radne procese i olakšava njihovo funkcionisanje. Ujedno zahteva i traži konstantno praćenje efikasnosti sistema uz neprekidne promene i unapređivanje svih elemenata radnog procesa i funkcionalnih jedinica.

Ključne reči: Kvalitet zdravstvene nege; Integrisani sistem menadžmenta; Indikatori kvaliteta zdravstvene nege; Organizacija i administracija; Zdravstvene usluge

Introduction

The term "management" is of English origin. It is difficult to define it fully, but perhaps it can be best formulated as a "control" of certain economic or social processes in order to achieve certain objectives through rational use of material and immaterial resources by applying the principles, functions

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Abbreviations

ICVDV – Institute of Cardiovascular Diseases of Vojvodina
ISO – International Standardization Organization
IQMS – Integrated Quality Management System
IMA – Integrated Management System

and management methods. Although there is no universally accepted definition of management, the well known expert on this issue P.F. Drucker provides the following definition in his article "Management Tasks, Responsibilities, and Practices" [1]: The management is independent of the ownership, position and power. In the system of management, there are four important concepts that significantly improved application of management in practice:

1. Scientific management
2. Administrative management
3. Management in the aspect of psychology and human relations
4. Management with the aspect of the science of behavior

The principles of "scientific management" are based on the introduction of science in the process of planning the production process, election of workers, their further education, and equal influence and responsibility of management (organ management) and workers in creation of efficient production processes [2]. "Administrative Management" is based on the improvement and reorganization of the system of mutual connections and processes in a company with exclusion of influence of the environment. An important contribution of this school and of one of its eminent representatives H. Fayol [3] is in his seeing the management as a universal process. "Management through the prism of psychology and human relations" was a very popular concept in the mid twentieth century. This approach says that the productivity could be increased significantly not in the form of wage increases, but through closer relationship of management (management body) and workers as well as through increased satisfaction of workers with their job and in their active participation in the processes of production and modifications of this process [4]. "Management in terms of the science of behavior" is a more modern concept that was developed in the second half of the XX century and it refers to increasing the efficiency of production process based on increasing the efficiency of human resources. To eliminate barriers to international trade in goods, services and information, it was necessary to harmonize national laws and standards in the field of quality management system. Therefore, in its "white paper" [5] the European Union (EU) pointed out the need to conform them by the year 1992. Even before that (in 1979), the Technical Committee of the International Organization for Standardization (International Standardization Organization – ISO) started to work on the harmonization of standards in the field of management on the basis of British Standard BS 5750, and in 1987 they issued a series of standards ISO 9000, and a revision of them in 2000 as series of standards ISO 9001. In our country it is officially designated as a series of standards ISO 9001, while in the European Union they are adopted as EN 29000. The fundamental role of these series of standards is to help organizations to improve the quality of work, communication, and competitiveness in domestic and international exchange.

Health care is one of the most important social institutions. Only a healthy individual can be happy and satisfied and can perform other socio-economic functions. Being the most important product of health care system, health must be achieved quickly and efficiently in order to enable a sick person to return to his/her daily and regular socio-economic activities as soon as possible and thus to contribute to themselves, their family, and the society as a whole.

The process of implementation of the Integrated Quality Management System (IQMS) and the results in one of the most important health care institutions of tertiary type – the Institute of Cardiovascular Diseases of Vojvodina (ICVDV) will be explained further in the text. The ICVDV is a tertiary health institution of high quality of work in the previous period, and it consists of the Department of Cardiology, Department of Cardiac Surgery and Technical Services. The main activities of the ICVDV are to:

1. Monitor and study the situation in the field of cardiovascular diseases,
2. Investigate, introduce and implement new methods of detection of diseases, treatment and rehabilitation, and to implement measures for improving health care in the area,
3. Perform complex diagnostics, treatment (including surgery) and rehabilitation of patients,
4. Perform education and training of health workers in their field of work,
5. Perform scientific research in the areas of prevention, treatment and rehabilitation in the field of cardiovascular diseases,
6. Provide technical and methodological assistance to health institutions in this field.

This study has been aimed at estimating the value of integrated management system applied at our Institute in order to raise the quality of treatment.

Material and Methods

Preparations for the introduction of ISO standards and the IQMS began in 2008. The initiative is mainly the result of many years of "Twining" cooperation between the ICVDV and the hospital "Alessandro Manzoni" from Lecco (Lombardy, Italy). That particular hospital had implemented the ISO 9000 standard even before 2000, with the accompanying standards of environmental protection and safety. During regular meetings with the representatives of that hospital, the modalities of cooperation in several areas were discussed, and since the beginning of the implementation of ISO standards it has been ranked as one of the most important roles in the areas of cooperation of these two institutions. The representatives of Lecco hospital suggested strongly that the implementation of ISO standards was necessary in order to carry out the reorganization of structures and personnel of in-hospital and out-hospital health services in the field of liability, which was one of the essential

items in terms of increased demands of patients for better and faster treatment and announced net spending on hospital and outpatient costs treatment to make a compromise, but not at the expense of the quality of treatment and the patient's satisfaction. The program of introduction and implementation of the IQMS at the ICVDV was introduced as an official document of the two-year period cooperation approved by the Government of Lombardy and the Government of Vojvodina. During the year of 2008 and the following year, about 40 employees of the ICVDV (having academic and secondary school education of different profiles) attended the courses for the implementation of ISO standards given by the lecturers from the Faculty of Technical Sciences (the Departtment of Industrial Engineering and Management - FTS IEM) [6] of the University of Novi Sad. During that period, the personnel from the ICVDV management that would be responsible for the introduction and implementation of the IQMS were slowly profiled. Motivations of management representatives of the IQMS and their associates were different but the overall goal for all of them was a better organization of work processes and the improvement of quality of treatment for patients. One example of motivation of management representatives of IMS was: "A patient from the Department of Cardiology was found to have significant left main stenosis during coronary angiography at the end of the morning shift. In this case, would it be the obligation of the interventional cardiologist to inform the doctor from the Department about the finding, or is it the opposite-should the doctor from the Department ask the interventional cardiologist about the finding in his/her patient?" Both aspects are logical. The doctor who found a significant and life threatening finding is expected to report it, but the doctor who is responsible for the patient should also be interested in his findings after the intervention. This is just one example illustrating that if problems are not systematically solved, they can result in detrimental effects on the patient. These situations and many others in the field of profession, organization, education, or scientific research, etc., require precisely defined practices and procedures in order to minimize inconsistencies either resulting from badly defined procedures or being the consequences of non-compliance with the above mentioned procedures.

That complex institution (the ICVDV) with a very broad spectrum of highly specialized professional, educational and scientific activities with about 560 employees of different profiles required a lot of work in the preparation and implementation of the IQMS. First of all, general concepts, which were supposed to determine the direction in which the further development of ICVDV would go, had to be defined. To that end, the following conclusions were made:

ICVDV mission

In prevention and treatment of cardiovascular disease the tendency is to excel and improve the quality of life and dignity of patients according to the highest standards.

ICVDV vision

To make the ICVDV one of Europe's most reputable hospitals of this type, as well as a leader in the Balkans and South East Europe in cardiology and cardiac surgery.

– The system of values

– Quality above all (Only the service of high quality guarantees successful performance)

– Professionalism (only a strict professional relationship guarantees fulfillment of patient's requests and demands of other interested parties)

– Trust (mutual trust between the ICVDV and patients is a necessary condition for success)

– Motivation (only motivated employees will participate consciously in the realization of the mission and vision of the ICVDV)

– Perfectionism (only fully developed solutions to the problems can be the basis for success)

– Optimism (only optimistic people have a chance for success)

– Perseverance (only those who do their best on their way to improvement can expect to succeed)

– Devotion (only employees devoted to their institution can help it to achieve its mission and vision)

– Ability to learn and to predict events (only those capable of continuous learning and prediction of events may provide survival and development of the ICVDV).

The IQMS [7] itself complies with the requirements of ISO 9001: 2008, which is general and has served as the basis. In addition, the extent and type of activities necessitated the introduction of environmental management system in compliance with the requirements of ISO 14001: 2005; health care system and safety at work in compliance with standard BS OHSAS 18001: 2007; and biochemistry laboratory management system in compliance with the requirements of ISO/IEC 17025: 2006.

Once the mission, vision, and values had been defined as permanent parameters of the role and functions of ICVDV, procedures had to be written for each functional unit or functionally different processes in the context of the ICVDV functioning. Because of the presence of two major functional units of the ICVDV each special in some way within its competence, but constituting an inseparable whole, all anticipated procedures were divided into two groups: the Procedures for the Department of Cardiology (CP), and Procedures for the Department of Cardiac Surgery (CSP) besides the general procedures (GP), which had 14 procedures and one instruction and the specific procedures (SP), which had 9 procedures and 2 instructions. The team assigned to write the procedures had first to become familiar with the organization of work of each department in order to gain insight into very complicated work process, and to make not more than 10-12 different procedures. The procedures had to include all segments of the organization and work, and be connected effectively with each other in some way.

Results

At the Department of Cardiology, 11 procedures and 5 special instructions for the operation in special situ-

ations (e.g. in cases of cardio-pulmonary resuscitation, or procedures for patients with ST segment elevation myocardial infarction outside Novi Sad, etc.) were written. A total of 7 procedures were written at the Department of Cardiac Surgery. While considering the needs for the size and number of procedures, parts of work processes that were identical or similar had to be sorted out and thus they represented the basis for writing a single procedure regardless of job title, organizational units or functions. The point was to make one good and logical functional unit with the lowest number of procedures (written records), and to distinguish and write specific procedures only in those processes of work which, by its system of functioning, were really different. During the writing process it was often observed that the work processes or work organization had shortcomings. They were often not noticed when the writing began, but were observed during the writing so the remarks or immediate changes were made by the participants during the creation of the document.

Each procedure was a functional integration of a specific work process while its writing had to be considered in a wider consensus and with team members, holders of management and in discussion with educators when introducing the IQMS. In this way, a valid document was obtained, which, nevertheless, had to be aligned with other procedures at the end of the process if they had some things in common in order to avoid repetition or redundancy. Upon completion, the procedures were thoroughly discussed by the team members, and had to be analyzed in the presence of representatives of management for the IQMS and their assistants. Only after such multiple analyses could it be clearly seen what the dimensions of functioning were in complex health institutions such as the ICVDV, in this case. The activity of writing the procedures or rules for work process and functioning, which are incorporated into documents, represents a very strong motivation to look at the process constantly and to make efforts to improve the health care of patients constantly and continuously (as well as other activities: scientific research; work in educational activities, and many other segments). The functioning of such a complex system as the ICVDV is never static. It is a "living organism" that requires constant adaptability and very important changes of different characters in short time intervals, with the aim of improving the quality of performance and efficiency and eventually increasing patients' satisfaction with treatment. For these reasons, many of the procedures and guidelines have been revised several times, which means that it was important to change the above mentioned documents as a result of permanent improvement of workflow and organization charts. Some changes were made to improve the quality of treatment, which was followed by regular analysis of patient's satisfaction with treatment measures (**Tables 1 and 2**). As a result of evaluating the quality of cardiac surgery treatment, and within the set quality goals, the "Vojvodina Score" model for calculating operative risk in surgical patients from this area was created [8, 9]. It has introduced some new input elements that characterize the operative cardiac surgery risk factors in these areas, which partly differ from the factors of operative cardiac surgery risk in other parts of the world. All written and mandatory procedures and guidelines approved by the manage-

Table 1. Patient's satisfaction with the admission and discharge services at index hospitalization (maximal mark 5)
Tabela 1. *Zadovoljstvo pacijenta uslugama prijema i otpusta tokom indeksne hospitalizacije (maksimalna ocena 5)*

Admission and discharge services during the index hospitalization *Usluga prijema i otpusta tokom indeksne hospitalizacije*	
1 General impression of the admission procedure/*Opšti utisak o proceduri prijema*	4.4
2 Staff courtesy/*Ljubaznost osoblja*	4.5
3 Waiting time at the counter/*Vreme čekanja na šalteru*	4.2
4 Explanation of procedures during admission/*Objašnjenje procedure tokom prijema*	4.3
5 Time to being accommodated in the room/*Vreme do smeštaja u sobu*	4.3
6 General impression at discharge/*Opšti utisak prilikom otpusta*	4.4

Table 2. Patient's satisfaction with physician's servicies during index hospitalization (maximal mark 5)
Tabela 2. *Zadovoljstvo pacijenata uslugama lekara tokom indeksne hospitalizacije (maksimalna ocena 5)*

Physician's services during index hospitalization/*Usluge lekara tokom indeksne hospitalizacije*	
1 Readiness to respond/*Spremnost da daje odgovore*	4.4
2 Explanations/*Objašnjenja*	4.3
3 Respect and kindness/*Poštovanje i ljubaznost*	4.4
4 The ability to diagnose/*Sposobnost dijagnostikovanja*	4.5
5 Thoroughness in testing/examining/*Temeljnost u ispitivanju*	4.5
6 Treatment successfulness/*Uspešnost lečenja*	4.4
7 Instructions at discharge/*Uputstva pri otpustu*	4.5
8 General satisfaction with services/*Opšte zadovoljstvo uslugama*	4.5

ment were posted on the internal internet site of ICVDV, and thus became available to those engaged in the processes described in these documents, who could gain an insight into their respective part of the organization or business activities. The documents placed on the site cannot be changed, but there are clearly defined procedures how they can be changed (proposal and request for modification of documents - procedures and/or instructions).

The Rules of IQMS, placed on the internal ICVDV site, is the final and main document which has replaced the previously described documents. This document describes the systematization, mission, vision, policy, organizational form, and structure of the ICVDV as a complex medical work organization. The document, which contains 66 pages with diagrams and tables, gives a valid insight into the functioning of the ICVDV in the present time and objectives of quality of work in future.

The Institute of Cardiovascular Diseases of Vojvodina has received the required accreditation very easily and for a long period of time (7 years).

Discussion

When speaking of the Integrated Management System (IMS), it represents the incorporation of different standards as well as a comprehensive management tool (management body) that connects all the elements of business system in a unique and comprehensive system of management processes in one organization in order to meet demands of shareholders and to achieve business goals in line with the vision and mission of specific organization. By the integration of management systems, besides other things, cost reductions can be achieved thanks to a better organization within the work organization, and to the general satisfaction of both employees and end-users of healthcare, which all together reflect a better business organization as a whole.

The health care system is one of the pillars of the social system and also a measure of its quality. In order to achieve high quality in the area of health and to do that in a shorter period of time with the least possible expenditure of human and material resources (efficiency and effectiveness), it is necessary to apply the IQMS in Health Care [10, 11].

The necessity of the application of IMS in health institutions of the Republic of Serbia

What characterizes the health care system of the Republic of Serbia [12] is that the financial budgetary resources for Health Care are fairly limited and are considered to be among the lowest appropriations in Europe (less than 300 Euros per capita per year). A large part of health care is provided in the form of primary, secondary and tertiary health care as a form of legal regulation of the health organization structure of our country that sees the health industry as non-profit. From all mentioned above it is clear that the range of IMS quality in such circumstances is limited and cannot count on additional or higher sources of funding.

So this is why questions are occasionally asked, such as if it is appropriate to implement the IQMS in health care organizations, and in the given circumstances, what the possibilities for management of health care organizations are to improve the quality of health services as the basic criteria of satisfaction and purpose for end-users (patients) [13–15]. In doing so, the quality of the social health services must be viewed through three major segments: 1. Degree of excellence, 2. Achieved level, 3. Satisfaction of specific needs of the system and users of health care services.

The authors believe that the medical activities should have a constant tendency towards improving the quality of health services irrespective of current possibilities of budget financing. It also reflects the role of modern management to improve and modernize the organization structure of health institutions which they run. The introduction of standards and the IQMS is essential for reorganization of business in health organizations at all levels of care. Only in this way can the quality of treatment and patients' satisfaction be improved, which must be the main motive for implementation of ISO standard in health care system.

Even those institutions which used to be the flagship of health must strive for further improvement in quality of treatment for patients a permanent premise.

Conclusion

In order to achieve high quality in the health area and to do that in the shortest time and with the least possible expenditure of human and material resources, it is necessary to apply integrated quality management system. The application of this system allows a realistic assessment of functioning quality of work processes but also asks for constant monitoring of system efficiency and allows the changes which can lead to its better functioning. In this way the quality of treatment is increased and the quality objectives in the future are improved; in addition, inconsistencies that must be corrected are better seen. This gives a constant pace necessary for better organization of all agents with lower costs which should eventually result in a higher quality of treatment. The integrated quality management system prevents "embracing" in the own values and requires action. The employees play a big role in all this, as well as the management which regularly monitors and analyzes the results achieved in relation to its objectives.

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CHEMOTHERAPY ANALYSIS IN MASSIVE TRANSFUSION SYNDROME

ANALIZA HEMOTERAPIJE U SINDROMU MASIVNE TRANSFUZIJE

Bratislav STANKOVIĆ and Goran STOJANOVIĆ

Summary

Introduction. Massive transfusion is defined as blood transfusion in quantities equal to or greater than the estimated patients' blood volume over a relatively short period of time (3-4 hours). The study was aimed at analyzing the application of chemotherapy in treatment of patients with acute massive bleeding and evaluating the results of hemostasis and platelet counts screening tests in the patients receiving massive transfusions. **Material and Methods.** Attempts were made to fully compensate hemostatic factors in 24 patients (14 male and 10 female, aged 23 to 76 years) with acute massive and uncontrolled surgical bleeding (polytrauma, abdominal aortic aneurysm, digestive tract bleeding as a result of a farina overdose, mortus fetus) over the five-year period, wherein a circulating patients' blood volume was compensated over a relatively short period of time. First the surgical bleeding was stopped. The objective of chemotherapy was the combined use of resuspended red blood cells, fresh frozen plasma, cryoprecipitates and the platelet concentrate in order to maintain the patients' normal circulating blood volume and blood pressure (systolic blood pressure ≥ 100 mmHg) with hemoglobin value higher than 100 g/l and the hematocrit above 0.30 l/l. **Results.** Transfusion treatment of 24 patients with acute bleeding consisted of an average of 16 to 18 units of resuspended red blood cells (ranging from 4,880 ml to 5,220 ml); fresh frozen plasma (980 ml to 1,220 ml); cryoprecipitates (an average of 10 to 15 units i.e. 500-750 ml) and concentrated platelets (approximately an average of 8 to 12 units i.e. 240 to 360 ml). **Conclusion.** In our study we have confirmed the pathophysiological mechanism shown in the available medical literature that after transfusion of a large red blood cell concentrate volume, dilutional coagulopathy develops, caused by a sharp drop in platelet count and the significantly reduced activity of unstable coagulation factors in the patient's circulation.

Key words: Blood Transfusion; Blood Component Transfusion; Treatment Outcome; Blood Coagulation Tests; Hemostasis; Platelet Count; Hemorrhage; Disseminated Intravascular Coagulation

Sažetak

Uvod. Masivna transfuzija se definiše kao transfuzija krvi u količinama jednakim ili većim od procenjenog volumena krvi bolesnika, u relativno kratkom vremenskom periodu (3-4 sata). Cilj istraživanja je analiza primene hemoterapije u tretmanu bolesnika sa akutnim masivnim krvarenjem i procena vrednosti rezultata skrining testova hemostaze i broja trombocita kod bolesnika koji su primili masivne transfuzije. **Materijal i metode.** Pokušaji potpune nadoknade hemostaznih činilaca kod 24 pacijenata (14 muškaraca i 10 žena, starosti od 23 do 76 godine) u petogodišnjem periodu, kod akutnog masivnog i hirurški nekontrolisanog krvarenja (politrauma, aneurizme abdominalne aorte, krvarenje iz digestivnog trakta kao posledica predoziranja farinom, fetus mortus), gde je nadoknađen jedan cirkulišući volumen krvi bolesnika, u relativno kratkom vremenskom periodu. Najpre je hirurški zaustavljeno krvarenje. Cilj hemoterapije bio je kombinovana primena resuspendovanih eritrocita, zamrznute sveže plazme, krioprecipitata i koncentrovanih trombocita, radi održavanja normalnog volumena cirkulišuće krvi i krvnog pritiska bolesnika (sistolni pritisak ≥ 100 mmHg) i koncentracije hemoglobina više od 100 g/l, vrednosti hematokrita više od 0,30 l/l. **Rezultati.** U transfuziološkom zbrinjavanju 24 pacijenta sa akutnim krvarenjem utrošeno je: 16 do 18 jedinica resuspendovanih eritrocita (prosečno 4 880 ml do 5 220 ml); zamrznute sveže plazme (prosrečno 980 ml do 1 220 ml); krioprecipitata (prosečno 10 do 15 jedinica, tj. 500−750 ml) i koncentrovanih trombocita (prosečno 8 do 12 jedinica, tj. 240 do 360 ml). **Zaključak.** U našoj studiji potvrdili smo patofiziološki mehanizam, prikazan u dostupnoj medicinskoj literaturi, da se posle transfuzije velikog volumena deplazmatisane krvi, razvila diluciona koagulopatija, uslovljena velikim padom broja trombocita i znatno sniženom aktivnošću labilnih činilaca koagulacije u pacijentovoj cirkulaciji.

Ključne reči: Transfuzija; Transfuzija derivata krvi; Ishod lečenja; Koagulacioni testovi; Hemostaza; Broj trombocita; Krvarenje; Diseminovana intravaskularna koagulacija

Introduction

Massive transfusion is defined as blood transfusion in quantities equal to or greater than the patient's estimated blood volume over a relatively short period of time (3-4 hours) or as a compensation of the total circulating patient's blood volume by transfusion of stored allergenic blood within 24 hours [1–4]. However, life saving of a patient with acute massive blood loss (30%-50% of total circulating blood volume) demands large blood quantities to be transfused over the shortest period of time. Such transfusions, though lesser than the total patient's blood volume, must be defined as massive ones because they can cause serious homeostatic disorders. Therefore, restricting the definition of

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Abbreviations

2,3 DPG – 2,3 diphosphoglycerate;
APTT – activated partial thromboplastin time
VMA – Military Medical Academy
PT – prothrombin time
TT – thrombin time
Hb – hemoglobin
Hct – hematocrit
CP – concentrated platelets
FFP – fresh frozen plasma
CRYO – cryoprecipitate
RES. ER – resuspended erythrocytes
GIT – gastrointestinal tract
DIC – disseminated intravascular coagulation

massive transfusion only to situations in which more than one (or more) volume of patient's circulating blood are compensated over 24 hours does not provide a complete definition of massive transfusion [1, 3, 4].

Massive transfusions are applied in the treatment of patients with hemorrhagic shock resulting from an injury, surgery or heavy bleeding (usually gastrointestinal or gynecological). It is well known that the storage of preserved blood at temperatures of 4± 2 °C does not stop metabolic and degradation processes, it just slows them down, causing the free hemoglobin, potassium, ammonia and lactic acid content increases in the blood, the disappearance of unstable coagulation factors activity, a decrease in the 2,3 diphosphoglycerate (DPG) or 2,3 DPG concentration and an increase in acidity, i.e. a decrease in the blood pH [1–5]. For these reasons, massive transfusion may be the cause of a number of disorders creating the massive transfusion syndrome: electrolytic and acid-based imbalance, impaired oxygen transport, hypothermia, disorders due to the presence of micro-aggregates and hemostasic disorder [6–14]. Another massive transfusion side effect worth mentioning is the post-transfusion depletion of coagulation factors, which may result in the unwanted development of hemorrhagic syndrome. After transfusion of a large volume of conserved (not fresh) blood or large amounts of red blood cells concentrate, a dilutional coagulopathy develops, caused by a sharp drop in the platelet count and the significantly reduced activity of unstable coagulation factors in the patient's circulation [14–17]. One of the most significant clinical manifestations of massive transfusion syndrome is bleeding, usually from the surgical wound, injury or puncture spot; it is rarely generalized with bleeding into the skin or mucous membrane [1, 4, 7].

According to some multidisciplinary studies [15–18], in hemorrhagic shock resulting from digestive tract bleeding, trauma or some other cause, the compensation of lost volumes typically begins with crystalloids and colloids, and then rapidly progresses through blood and chemo products transfusion (chemotherapy). The compensation of large volume blood loss can cause coagulopathy that can be difficult to manage in case of uncontrolled bleeding. Coagulopathy during massive transfusion is a multifactorial pathophysiological mechanism that occurs due to hemodilution, hypothermia, the use of fractionated blood products and disseminated intravascular coagulation (DIC). Maintaining body temperature within normal limits is the first line of an effective strategy to improve hemostasis during massive transfusion. Treatment includes maintaining adequate tissue perfusion, correction of anemia and the use of hemostatic blood products [18–23].

The results of screening tests in massive transfusion syndrome show thrombocytopenia, with the extension of the activated partial thromboplastin time (APTT) and prothrombin time (PT) and thrombin time normal values (TT). It is necessary to monitor the hemostasis through laboratory testing during massive transfusion, and assess the frequency of further testing and the need for substitution transfusions therapy according to the results. It is often necessary to differentiate whether the hemorrhagic syndrome is a consequence of massive transfusion or DIC. In the massive transfusion syndrome, unlike the DIC, TT and fibrinogen concentrations are within the normal limits [4, 6–8].

In acute massive and surgically uncontrolled bleeding, where one patient's circulating blood volume has been compensated, the attempts to complete compensation of hemostatic factors in order to achieve adequate hemostasis are not useful. It is first necessary to stop the bleeding surgically. The combined application of stored whole blood, red cell preparations, colloid or crystalloid infusion solutions should maintain normal circulating blood volume and blood pressure of patients, as well as the hemoglobin concentration value (Hb) above 100 g/l or hematocrit (Hct) higher than 0.30 l/l. If the compensation of the lost blood is 0.2 l/hour or more, surgical bleeding is likely to be present, which would firstly require surgical care of patients. The application of hemostatic chemo products is indicated when the bleeding is caused by deficiency of hemostasis factors and not by surgical bleeding from an injured large blood vessel. Hemostasis deficit should be confirmed not only clinically, but also with laboratory findings. Thereby, DIC is the most complex therapy; out of hemostatic chemo products concentrated platelets (CP) (one unit/10 kg), fresh frozen plasma (FFP) (12 ml/kgbm) or cryoprecipitate (CRYO) (1-1.5 units/kgbm 10) should be applied, along with other medication therapy [1, 4, 7, 8]. It was previously thought that acute massive blood loss required compensation with the whole fresh blood exclusively (for the purpose of preventing or correcting hemostatic disorders, in addition to the erythrocytes compensation) [4, 14]. However, the introduction of targeted chemotherapy has changed this attitude in compliance with the concept that the use of only fresh whole blood ("uncooled" blood transfused immediately after taking it from a donor) is not only insufficiently effective in treatment of most patients, but also irrational. That is why the modern aimed chemotherapy recommends erythrocyte preparations, especially resuspended erythrocytes (RES. ER) [1, 4, 15–18].

According to some foreign studies [24], transfusion management of polytraumatised patients during peacetime should begin with the replacement

of lost blood volume with RES.ER until the patient is transported to a higher level of care (hospital inpatient facilities). Simultaneous infusion of FFP is proposed before surgical intervention. According to the recommendations of this study [24], the ratio of transfused RES. ER and FFP volume should be 1:1. Experience in the management of war injuries is similar, suggesting that RES. ER and FFP simultaneous compensation should be carried out in the ratio of 1:1 in supportive transfusion therapy [24]. Other studies [25] recommend transfusion trigger for the restoration of the lost circulatory volume with RES. ER transfusions at a dose of 40-60 ml/kgbm until reaching the concentration of Hb 100 g/l and Hct 0.30 l/l value [24, 25].

Further clinical trials are required for faster diagnosis and better definition of difference between the harmful effects caused by acute massive blood loss and massive blood transfusion, as well as rational and timely use of certain chemo products containing the necessary hemostatic factors [15–18].

Based on the experience gained in some foreign studies [24–26], an operative hypothesis was set up: "If in patients with acute massive blood loss immediate measures are applied as early as possible during transportation (surgical hemostasis, intravenous solutions infusion and oxygen dispensation), the intravascular volume will be preserved and will prevent the development of irreversible hypovolemic shock and DIC".

This article was aimed at analysing the application of chemotherapy in the treatment of patients with acute massive bleeding and evaluating the hemostatic and platelet counts screening tests results in the patients receiving massive transfusion.

Material and Methods

Transfusion management of 24 patients with acute massive and uncontrolled surgical bleeding (14 men and 10 women, aged 23 to 76 years) was followed through retrospective analysis at the "Bežanijska Kosa" Clinical Hospital Center in Belgrade during the five-year period. This study included 10 patients with polytrauma, 8 patients who had undergone surgery for abdominal aortic aneurysm, 4 patients with gastrointestinal (GIT) tract bleeding as a result of a farina overdose and two women with uterus revision after a stillborn fetus ("fetus mortus") (**Table 1**).

First, all these patients had adequate surgery and surgical hemostasis in order to prevent further uncontrolled bleeding. Due to the forthcoming hemorrhagic shock, a circulating blood volume of all the patients was compensated by certain chemo products in a relatively short period of time (3-4 hours). The chemotherapy goal was the combined use of RES. ER; FFP; CRYO and CP, thus maintaining the patients' normal circulating blood volume and blood pressure (systolic blood pressure of 100 mmHg) until they reached the value of Hb 100 g/l, i.e. value of Hct 0.30 l/l.

We performed a hemogram test [Hb (g/l) and Hct (l/l value)] and controlled the values of the hemostatic screening tests results in the patients who had received massive transfusions [PT which measures the plasma clotting time in the presence of tissue thromboplastin optimal concentrations, demonstrating the effectiveness of the extrinsic clotting pathways; activated partial thromboplastin time (APTT) which measures the clotting time after the contact factors activation, when tissue thromboplastin has not been added, so this test measures the activity of the intrinsic pathways of coagulation, while TT measures the clotting time after adding thrombin to plasma, influenced by the fibrinogen concentration and quality, fibrinogen, fibrin and heparin degradation products; the fibrinogen concentration was measured and protamine sulfate test done] [19]. In order to prevent the SEC, hemostasis test was repeated every 8 hours in the patients with massive transfusion syndrome.

Statistical parameters were used to calculate the mean values and percentage (%) ratio of the measured parameters, and the results were shown in tabular form and in the columnar graph.

Results

During the transfusion management of 24 patients with acute bleeding, the following materials were used on average: 16 to 18 RES. ER. units (approxi-

Table 1. Average consumption of certain chemo products in patients with massive transfusion
Tabela 1. *Prosečan utrošak pojedinih hemoprodukata kod pacijenata sa masivnim transfuzijama*

Pathological condition *Patološko stanje*	Number of patients *Broj pacijenata*	Average chemo products consumption (ml) *Prosečan utrošak hemoprodukta (ml)*			
		RES.ER./*RES.ER.*	FFP/*ZSP.*	CRY/*KRIO.*	PC/*KT.*
Polytrauma/*Politrauma*	10	2.050130	41090	210110	10050
Abdominal aortic aneurysm *Aneurizma abdo-minalne aorte*	8	1.650110	33080	17080	8040
GIT bleeding/*Krvarenje iz GIT-a*	4	87050	17040	9040	4020
Fetus mortus/*Fetus mortus*	2	41030	7030	3020	2010
Total/Average *Ukupno/Prosečno*	24	4.880320	980240	500250	240120

Legenda: RES.ER. – Resuspendovani eritrociti; *ZSP. – Zamrznuta sveža plazma*; *KRIO. – Krioprecipitat*; *KT. – Koncentrovani trombociti*; *GIT – Gastrointestinalni trakt*

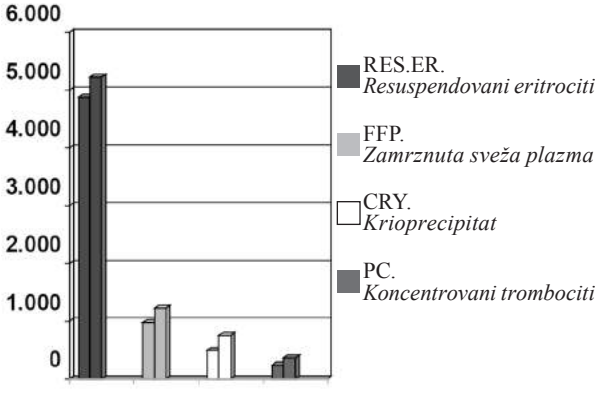
mately 4,880 ml to 5.220 ml); FFP on average 4 to 5 units, i.e. 980 ml to 1220 ml); CRYA (on average 10 to 15 units, i.e., 500 to 750 ml) and CT (on average 8 to 12 CT units or 240 to 360 ml). Transfusion management is shown in **Table 1** and the **Graph 1**.

According to the laboratory analyses, the number of platelets decreased in all patients who had received massive transfusions, being 3.2 to 12 times lower compared to the platelet number in the emergency room (70 x 10^9/l up to 550 x 10^9/l), and after massive transfusion, the platelet count ranged from 10 x 10^9/l to 150 x 10^9/l. Fifteen patients had lower platelet count of 55 x 10^9/l after receiving massive transfusions **(Table 2, Graph 2)**.

The results of hemostasis screening tests were abnormal in 68.6% patients, as follows: PT, PTT and TT were extended in 59.5%, in 23.1% and in 42.5% of the patients, respectively. Fibrinogen was reduced in 23.1%, and protamine sulfate test was positive in 38.3% of patients. Hemostasis test results indicated disseminated intravascular coagulation in 5 (20.83%) patients **(Table 3, Graph 3).**

Discussion

Trauma is the major death cause in young adults from 1 to 44 years of age. According to the World Health Organization (WHO) data [25], it is estimated that 5 million people die from injuries worldwide each year and the mortality rate is 83.7 per 100,000 inhabitants (traffic accidents and war injuries are particularly on the increase); therefore, the dramatic increase in the number of polytraumatized patients is expected until 2020. Up to 40% of such patients die due to circulatory shock as a result of acute blood loss. In addition to the surgical control of bleeding and adequate volume of resuscitation with the restoration of circulatory volume, adequate volume of chemo products (blood and/or blood products) is crucial for the survival of polytraumatized patients [24–26].



Graph 1. Average consumption of certain chemo products in transfusion management of 24 patients with massive transfusion
Grafikon 1. *Prosečan utrošak pojedinih hemoprodukata u transfuziološkom zbrinjavanju 24 pacijenata s masivnim transfuzijama*

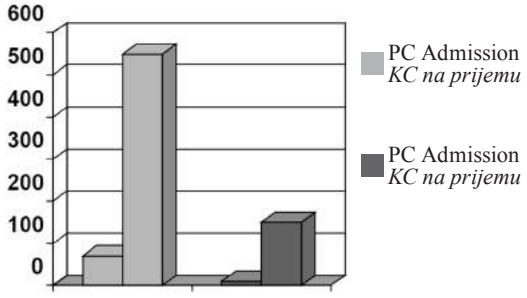
The most common causes of coagulation disorders in the patients with massive bleeding or massive transfusions are: coagulopathy due to loss of blood type, dilutional coagulopathy, consumptive coagulopathy, hyperfibrinolysis, acidosis, hypothermia, anemia and electrolyte imbalance [26].

According to recent studies [18, 21, 22], massive transfusion is defined as the entire volume of circulating blood restoration within 24 hours or 50% of blood volume compensation over 3 hours. In situations of acute bleeding, a definition based on the clinical practice experience is often used, which involves transfusion of 4 or more red cell units within one hour [23].

It is characteristic for massive transfusion that the total volume of transfused blood (required for adequate compensation) and the transfusion speed can potentially overcome the possibilities of compensatory mechanisms to maintain homeostatic balance in the patient's blood [1, 4, 16, 18].

In the patients with acute massive blood loss, it is necessary to take urgent measures during transport (surgical hemostasis, intravenous solutions infusion and oxygen administration) in order to maintain intravascular volume and prevent the development of irreversible hypovolemic shock [1, 4, 16].

The experiences of the first author of this study and a group of authors from the Military Medical Academy (VMA) in Belgrade [27, 28] have shown positive results in the survival and rescue of intraoperative autologous blood, as well as simultaneous intraoperative reinfusion of autologous blood recovered from uncontaminated hematothorax using "Pleur-evac system" in 25 wounded persons. They were classified into the fourth category of urgency ("moribund") after triage in a field hospital in war-affected areas in the former Yugoslavia (1991). During helicopter transport from a field hospital to the VMA, intraoperative autologous blood salvage was performed, as well as simultaneous reinfusion of autologous blood intraoperatively recovered from uncontaminated hematothorax using "Pleurevac system" [27]. These urgent measures increase the patients' survival rate. Acute loss of large amounts of blood always threatens patients' lives directly and is an indication for massive blood transfu-

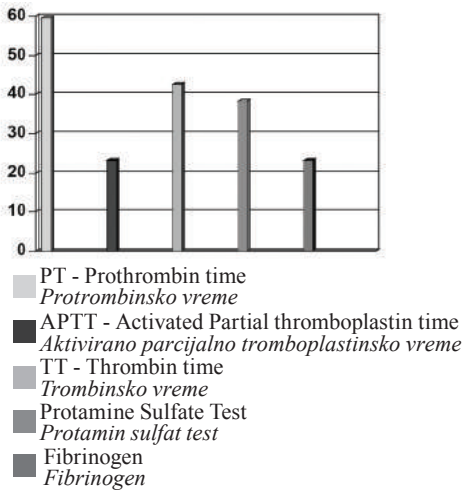


Graph 2. Patients' platelet count (10 x 10^9/l) on admission and after receiving massive transfusions
Grafikon 2. *Broj trombocita (10 x 10^9/l) kod pacijenata na prijemu i nakon primanja masivnih transfuzija*

Table 2. Changes of platelet count (10 x 10^9/l) on admission and after massive transfusions
Tabela 2. *Kretanje broja trombocita (10 x 10^9/l) na prijemu i nakon masivnih transfuzija*

Pathological condition *Patološko stanje*	*Number of patients* Broj pacijenata	Platelet count *(10 x 10^9/l)/Broj trombocita*	
		On admission *Na prijemu*	After massive transfusions *Nakon masivnih transfuzija*
Polytrauma/*Politrauma*	10	5 (50 - 53 x 10^9/l)	5 (10-15 x10^9/l)
		3 (52 - 55 x 10^9/l)	3 (60 – 130 x 10^9/l)
		2 (450 – 550 x 10^9/l)	2 (350 – 470 x 10^9/l)
Abdominal aortic aneurysm *Aneurizma abdominalne aorte*	8	4 (53 -54 x 10^9/l)	4 (45 – 52 x 10^9/l)
		3 (460 – 530 x10^9/l)	3 (360 – 510 x x10^9/l)
		1 (330-440 x 10^9/l)	1 (280 – 360 x 10^9/l)
GIT bleeding/*Krvarenje iz GIT-a*	4	3 (380 – 450 x 10^9/l)	3 (320 – 430 x 10^9/l)
		1 (320 – 440 x 10^9/l)	1 (280 – 410 x 10^9/l)
Fetus mortus/*Fetus mortus*	2	2 (51-54 x 10^9/l)	2 (35 – 52 x 10^9/l)

GIT - gastrointestinalni test



Graph 3. Results of chemostatic screening tests expressed in percentage (%) relation with pathological findings of the PV, APTT, TT and fibrinogen concentration
Grafikon 3. *Rezultati skrining testova hemostaze izraženi u procentualnom (%) odnosu sa patološki nalazima PV, APTT, TV i koncentracije fibrinogena*

sion. Impediment of surgical hemostasis reduces the survival rate of the bleeding patients' significantly. Prolonged uncontrolled bleeding causes death in 41% of traffic accident injuries. Heterogeneity of patients whose lives are saved by massive transfusion requires an individual approach with no room for rigid standard protocols [1].

All 24 patients in our study sample who have received massive transfusions showed the signs of thrombocytopenia (from mild to very severe), which was of a pathological or a dilutional-type. According to the laboratory analysis, all patients had decreased platelet count after massive transfusion, which was 3.2 to 12 times lower than the number of platelets in the emergency room (70 x 10^9/l to 550 x 10^9/l), while after massive transfusions, the platelet count ranged from 10 x 10^9/l to 150 x 10^9/l. Fifteen patients had lower platelet count of 55 x 10^9/l after having received massive transfusions. Drastic drop in the platelet count after massive transfusion was compensated to the patients with the available number of platelet units in the Clinical Hospital Center "Bežanijska Kosa" Transfusion Service.

Table 3. Number of patients with reduced chemostatic test values after massive transfusions
Tabela 3. *Broj pacijenata sa sniženim vrednostima testova hemostaze nakon masivnih transfuzij*a

Pathological condition *Patološko stanje*	Number of patients *Broj pacijenata*	Reduced chemostatic test values after massive transfusions *Snižene vrednosti testova hemostaze nakon masivnih transfuzija*				
		PT	APTT	TT	Fibrinogen	Positive protamine sulphate/*Test pozitivan protamin sulfat test*
Polytrauma/*Politrauma*	10	8 (33.3%)	3 (12.5%)	5 (20.1%)	3 (12.5%)	4 (16.7%)
Abdominal aortic aneurysm *Aneurizma abdominalne aorte*	8	6 (25%)	2 (8.3%)	3 (12.5%)	2 (8.3%)	2 (8.3%)
Git bleeding/*Krvarenje iz git-a*	4	1 (4.2%)	1 (4.2%)	1 (4.2%)	1 (4.2%)	2 (8.3%)
Fetus mortus/*Fetus mortus*	2	1 (4.2%)	-	1 (4.2%)	-	1 (4.2%)
Total number/ratio in % *Ukupan broj/% odnos*	24 (100%)	14 (59.5%)	6 (23.1%)	10 (42.5%)	6 (23.1%)	9 (38.3%)

Legenda: PT - protrombinsko vreme, APTT - aktivirano parcijalno tromboplastinsko vreme, TT - trombinsko vreme

Our study as well as other larger clinical ones [21–26] have proved the main postulate that a drastic fall in the platelet count (apparent thrombocytopenia) occurs primarily as a result of consumptive coagulopathy after massive transfusions.

The deficit of coagulation factors was adequately compensated by providing sufficient volume of FFP and CRYA.

Screening test results reported in many foreign [1, 15, 17, 21–26] and national medical publications [4, 16, 18] showed that massive transfusion syndrome, in addition to the reduced number of platelets, leads to the extension of the APTT and PT and normal TT values. The results of our tests are in accordance with the above findings. For example, the hemostasis screening tests results were found to be pathological in more than two thirds (68.6%) of patients, and the most common disorder was prolonged PT in almost 60% of patients, and APTT and TT was prolonged in about a quarter of the studied patients (23.1%), and almost half of patients (42.5%), respectively. The fibrinogen concentration values were reduced in almost a quarter of the studied patients (23.1%), while a protamine sulfate test was positive in 38.3% of patients. Although screening tests for hemostasis were performed every eight hours after massive transfusion, one-fifth (20.83%) of patients developed DIC.

An attempt was made within this study during the five-year period to monitor and analyze chemotherapy with massive transfusion syndrome in a small group of patients. The opportunities in supportive therapy for massive hemorrhage were limited in our conditions. The aim of this study was to compare our experience in the analysis of chemotherapy in massive transfusion syndrome with international multicenter studies that included a much larger number of patients with massive transfusion syndrome, had access to a wide range of chemo products in supportive therapy, and had done more detailed laboratory tests in order to monitor hemostatic status and the clinical status of patients so as to prevent hemorrhagic shock and DIC [1, 15, 17]. Nevertheless, test parameters recorded in our patients were crucial and sufficient for the evaluation of hemostasis, the normalization of which is largely determinable for the chemotherapy effectiveness.

The shortcomings of our study are that in a retrospective analysis "limited" medical documentation was used and inadequately managed (without chemotherapy protocols at the wards and departments, without complete lists of blood transfusion and/or chemo products and with an incomplete registration of adverse effects of chemotherapy, i.e. non-compliance with the fundamental legal principles of hemovigilance prescribed by the "Law on Transfusiology" [20]. One of the shortcomings of this study is that it followed a small group of patients with massive transfusion syndrome over a short period of time and at only one medical center in Belgrade, unlike foreign multidisciplinary studies which include several decades of research with a greater number of patients at several medical institutions.

For these reasons, further research should be undertaken over a longer period of time, which would involve several hospital centers in the Republic of Serbia and analyze chemotherapy outcome in a larger sample of patients with massive transfusion syndrome. A wider range of laboratory tests should be applied and all necessary quantities of chemo products should be used in transfusion management of patients in order to prevent hemorrhagic shock and DIC.

Conclusion

This study has confirmed the pathophysiological mechanism found in available medical literature that dilutional coagulopathy, caused by a sharp drop in platelet count and the significantly reduced activity of unstable coagulation factors in the patient's circulation, develops after large volumes of red blood cell concentrate transfusion. Due to the rapid decline in the number of platelets, the majority of patients developed uncontrolled bleeding from the surgical wound, injury or puncture spot, which is rarely of the generalized type with bleeding into the skin or mucous membranes.

The results of screening tests obtained in our study are consistent with the findings reported in international studies, and they have shown that massive transfusion syndrome includes extended activated partial thromboplastin time and prothrombin time and in most cases the normal value of the thrombin time in addition to a reduced number of platelets.

The aims of our study have been fully accomplished, and the administration of chemotherapy in treatment of the patients with acute massive bleeding over the five-year period has been completely analyzed. In addition, the values of the hemostatic and platelet counts screening test results obtained for the patients who received massive transfusion have been thoroughly assessed.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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OTOLOGICAL MANIFESTATIONS OF TURNER SYNDROME: CLINICAL AND RADIOLOGICAL FINDINGS

OTOLOŠKE MANIFESTACIJE TARNEROVOG SINDROMA: KLINIČKI I RADIOLOŠKI NALAZI

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Summary
Introduction. Turner syndrome is a chromosomal abnormality where all or a part of one of the X chromosomes is absent or it has other abnormalities. Besides characteristic abnormalities of short stature and infertility, women with Turner syndrome have increased risks for tumors of the central nervous system, especially meningioma and an otologic disease. Meningioma involving the middle ear is extremely rare, and this condition has never been published in association with Turner syndrome. **Case Report.** We present an otologic manifestation associated with other abnormalities in a patient with Turner syndrome and discuss diagnosis and possible treatment options. **Conclusion.** Multidisciplinary team approach is essential in these patients in order to evaluate their vulnerability and define therapeutic priorities.
Key words: Turner Syndrome; Meningioma; Ear, Middle; Diagnosis; Radiology; Otolaryngology; Patient Care Team; Watchful Waiting

Sažetak
Uvod. Tarnerov sindrom je hromozomski poremećaj gde jedan X hromozom nedostaje delimično, ili u celini, ili ima druge abnormalnosti. Pored karakterističnih abnormalnosti u vidu niskog rasta i steriliteta, žene sa Tarnerovim sindromom imaju povišen rizik za nastanak tumora centralnog nervnog sistema, posebno meningeoma i otoloških bolesti. Meningeom koji zahvata srednje uvo je izuzetno retka pojava, a ovo stanje do sada nije objavljeno kod Tarnerovog sindroma. **Prikaz slučaja.** Predstavljamo otološku manifestaciju udruženu sa drugim anomalijama kod pacijenta sa Tarnerovim sindromom i razmatramo mogućnosti dijagnostike i lečenja. **Zaključak.** Multidisciplinarni timski pristup je neophodan kod ovih pacijenata radi procene njihove ugroženosti i definisanja terapijskih prioriteta.
Ključne reči: Tarnerov sindrom; Meningiom; Srednje uho; Dijagnoza; Radiologija; Otologija; Multidisciplinarni tim; Praćenje toka bolesti

Introduction

Turner syndrome was first described by Henry Turner in 1938. It is a chromosomal abnormality where all or a part of one of the X chromosomes is absent or it has other abnormalities [1]. The incidence of Turner syndrome (TS) is approximately 1:2000 live female births [2]. There are characteristic abnormalities, such as short stature, infertility, congenital heart disease, hypothyroidism, diabetes, vision problems, hearing impairments, and many autoimmune diseases. Women with TS have increased risks for tumors of the central nervous system (CNS), especially for meningioma [3]. In addition, an otologic disease is an important characteristic in TS [4]. It is mainly sensorineural hearing loss or otitis media. Meningioma involving the middle ear is an extremely rare in general [5].

In this paper, we present otological and radiological findings in a patient with TS and discuss necessary diagnostic procedures and possible treatments.

Case Report

A 44-year-old woman with TS was referred to the Clinic of Otorhinolaryngology with intermittent purulent, fetid and sanguineous discharge from the left ear and hearing loss lasting for more than three years. Otomicroscopy revealed a voluminous, soft mass in the left external ear canal (**Figure 1**). Enterobacter and Klebsiella spp. were isolated. Audiometry showed mixed hearing loss in the right ear and deafness in the left one. There were no neurological symptoms or deficits. High resolution computed tomography (CT) of temporal bones demonstrated hipodense mass (35-49 HU) in the left external ear canal, middle ear, antrum and mastoid air

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Abbreviations

TS – Turner syndrome
CT – computed tomography
MRI – magnetic resonance imaging
CNS – central nervous system

cells without bony destruction. Magnetic resonance imaging (MRI) showed an enhanced infiltration of left middle cranial fossa dura with infiltration of tentorium and mild compression of temporobasal brain with propagation in the left middle ear and the cavernous sinus and surrounding the left internal carotid artery (**Figure 2**). Angiography of cerebral blood vessels showed normal vascularity and excluded glomus tumors. Imaging characteristics were considered as a meningioma of the skull base with propagation in the left middle and external ear. The biopsy was performed from the mass protruding in the external auditory canal. Patohistological analysis revealed inflammatory granulation tissue.

This patient with TS had also hypothyroidism, microcytic hypochromic anemia and osteoporosis. Echocardiography showed persistent left vena cava superior and mild mitral and tricuspid regurgitations. During hospitalization the patient was treated with antibiotics for the infection of the middle and external ear. A multidisciplinary team consisting of otorhinolaryngologist, neurosurgeon, cardiologist and endocrinologist decided to repeat radiologic investigations after a year without any surgical intervention because she had no neurological deficits and hearing loss in the left ear was irreversible. It should also be noted that this decision was taken because of the high risk for surgery. A year later, otomicroscopic and MRI findings were the same. Again it was decided to adopt "wait and scan" policy.

Discussion

The authors presented a middle-aged woman with TS having the symptoms of chronic ear disease and unclear otomicroscopic and CT findings of a mass in the left external and middle ear without bone destruction. MRI findings were interpreted as a meningioma of the left middle skull base dura with propagation in the left, middle and external ear.

A middle ear disease, which affects 50-85% of girls and woman with TS, usually starts in early childhood and results in recurrent suppurative otitis media, serous otitis media, chronic suppurative otitis media and cholesteatoma [6]. The frequency of ear infection decreases with age [7]. Middle ear infections and hearing impairment in TS develop due to growth disturbances during development and delayed cell cycle caused by chromosomal aberrations per se and not only due to the specific X chromosome deletion [6]. A middle ear disease in TS is recurrent and leads to multiple surgical procedures [8]. Dhooge et al. [4] advocated careful follow-up during early childhood of girls with TS to prevent sequelae.

In addition, the patients with TS have increased risks for tumors of the CNS, especially for a meningioma and these risks might be associated with genetic and hormonal factors or effects of given hormonal treatments [3].

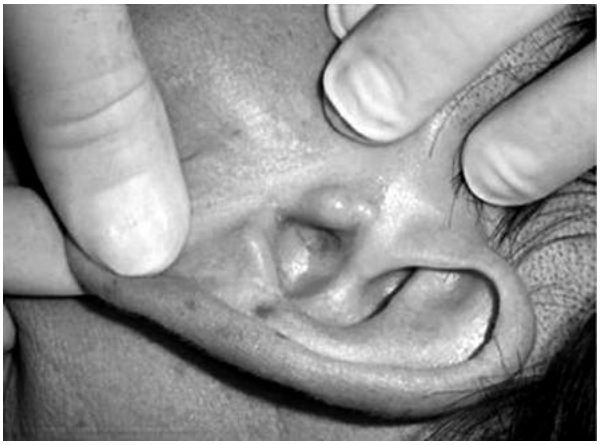

Figure 1. Macroscopic appearance of the tumor mass in the external auditory canal
Slika 1. *Makroskopski izgled tumorske mase u spoljašnjem slušnom kanalu*

Ayache et al. [9] reported ten patients presenting with signs of serous otitis media whose imaging revealed a temporal meningioma involving the middle ear. They recommended MRI for all cases with indirect signs of meningioma on CT scan. MRI with contrast typically shows an enhancing, dural-based,

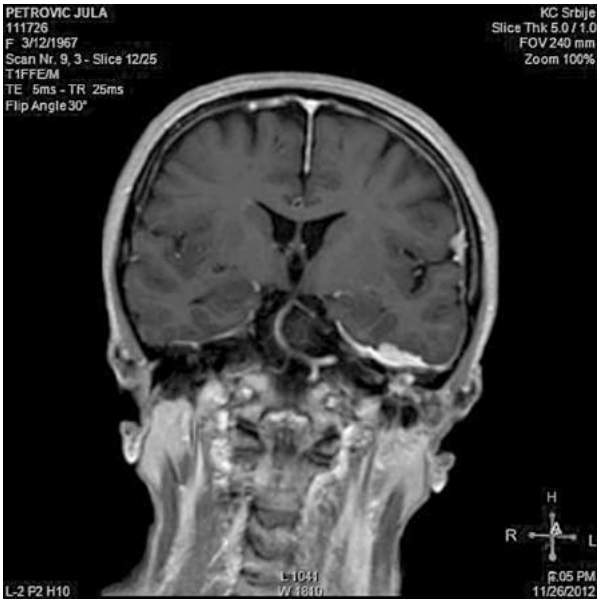

Figure 2. Coronal magnetic resonance imaging shows tumor mass of middle cranial fossa dura with propagation to the cavernous sinus and the left internal carotid artery without infiltration
Slika 2. *Nalaz koronalne magnetne rezonancije pokazuje tumorsku masu dure srednje lobanjske jame sa širenjem na kavernozni sinus i unutrašnju karotidnu arteriju sa leve strane, bez infiltracije*

soft tissue mass, while CT shows a hyperostotic bone reaction and a hairy aspect of margins of the affected bone.

Shihada et al. [5] presented three cases of skull base meningiomas mimicking otitis media diagnosed by CT in conjunction with MRI. Histology is not necessary for the diagnosis of meningioma.

Meningiomas are mostly benign tumors and the classical approach to meningioma treatment is surgical excision with good prognosis [10]. Fractionated radiotherapy or stereotactic single-dose radiosurgery is also recommended in selected cases. Hormonal therapy with progesterone antagonists and chemotherapy may be considered in cases of unresectable meningioma or where surgery or radiotherapy has failed [11]. Yano et al. [12] suggested conservative treatment with close follow-up to avoid surgery related morbidity in the patients with asymptomatic meningiomas. Ayache et al. [9] reported that conventional middle ear procedures were inefficient in treating a meningioma with otologic manifestations. Shihada et al. [5] suggested "wait and scan" policy in cases of neurologically asymptomatic patients and when treatment modalities carry a significant risk of morbidity.

In the presented case, the initial symptoms were otogenic complications due to chronic otitis media and that was the reason for the hospital treatment and urgent diagnosis. During further examination, radiological findings suggested the skull base meningioma with the middle ear propagation. However, the possibility of surgical treatment was limited because of a high risk for vital function. The multidisciplinary team concluded that there were comorbidities compatible with surgery, but there were no neurological deficits, hearing loss was irreversible and secondary infections of tumor in the external and middle ear was curable with antibiotics. The complete resection of the tumor was impossible without serious injury of neuronal and vascular structures. It has been decided to follow up this woman with TS with yearly scans.

Conclusion

The multidisciplinary team approach is essential in patients with Turner syndrome. The first signs of skull base meningioma were presented as chronic otitis media. Proper imaging diagnostics is necessary for the right diagnosis and treatment. Due to a high risk of surgical treatment in the patients with Turner syndrome, wait and scan policy is the most advocated.

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HIDRADENITIS SUPPURATIVA: A CASE SERIES OF EIGHT PATIENTS

HIDROADENITIS SUPURATIVA: SERIJA OD OSAM PACIJENATA

Momčilo STOŠIĆ and Igor STOJANOVIĆ

Summary

Introduction. Hidradenitis suppurativa is a persistent, inflammatory and recurrent disease of the hair follicles which, in time, results in ugly scars. Inflammation and obstruction of channel of sweat glands used to be thought to be the basis of this disease. **Case Reports**. This paper presents the cases of 8 patients operated on in the past 3 years. A possibility of an oversight in making the diagnosis, as well as an underestimation in the treatment should be taken into consideration. In addition to surgical methods the authors discuss other therapeutic possibilities taken from the available literature. This is a retrospective analysis of the surgical findings. The treatment was multidisciplinary but the surgical excision was the only option which provided healing without recurrence so far. In our series the excision could be done to the fascia or to leave a thin layer of adipose tissue. The reconstruction could be achieved by healing per secundam, by autologous skin or artificial graft, or by narrowing the wound with a skin portion. Our results achieved by stage, secondary stitches and rotation portions were satisfying. **Conclusion.** In case of advanced disease only surgery can lead to healing. We performed late surgery because the disease was diagnozed in advanced stages according to Hurlay. Wide excision was done in all our patients. The reconstruction was achieved with rotational flap in 3 patients, the wound healing per secundam in 4 patients and with primary suture in one patient. Our patients did not have recurrences of the primary disease. The disease was combined in one case with perianal fistula.

Key words: Hidradenitis Suppurativa; Hair Follicle; Sweat Glands; Surgical Procedures, Operative; Wound Healing

Sažetak

Uvod. Hidroadenitis supurativa je uporno, zapaljenjsko, rekurento, oboljenje folikula korena dlake koje se lako prenosi na znojne žlezde što zajedničko zapaljenje vremenom dovodi do ružnih ožiljaka. Zapaljenje i opstrukcija kanala znojnih žlezda je u osnovi ovog oboljenja. **Prikaz slučajeva.** Kroz rad prikazujemo osam operisanih bolesnika u poslednje tri godine. Mogućnost previda u postavljanju dijagnoze kao i potcenjivanje u tretmanu je prisutno. Uz operativne metode prikazujemo i pregled drugih terapijskih mogućnosti iz literature kroz metod retrospektivne analize operativnih podataka i intervjua sa bolesnicima. Poslednjih godina je otkriveno mnogo podataka o etiologiji bolesti. Lečenje je multidisciplinarno, ali hirurška ekscizija je, kao što prikazuju naši rezultati, jedina opcija koja obezbeđuje izlečenje bez recidiva. Ekscizija se može izvesti do fascije ili uz ostavljanje tankog sloja masnog tkiva (bolje prihvatanje transplantanta ili režnja), a rekonstrukcija može biti zaceljenjem per sekundam, autolognim kožnim ili arteficijalnim graftom, ili suženjem rane kožnim režnjem. Naši rezultati etapnim, sekundarnim šavovima i rotacionim režnjevima su zadovoljavajući. **Zaključak.** Kad govorimo o uznapredovalom stadijumu bolesti, samo hirurgija obezbeđuje izlečenje. Operativni tretman je široka ekscizija što smo primenjivali sa nekim od prikazanih metoda zatvaranja rane. Mogući su recidivi, a tada se praktikuje reekscizija. Naši bolesnici nisu imali recidive primarne bolesti. Hidroadenitis supurativa može biti udružena sa perianalnim fistulama, pilonidalnim sinusom, imunosupresivnim stanjima i drugim sindromima. Mi smo imali jedan slučaj udružen sa fistulom.

Ključne reči: Hidradenitis suppurativa; Folikul dlake; Znojne žlezde; Operativne hirurške procedure; Zarastanje rane

Introduction

In case of hidradenitis suppurativa (HS) most surgeons think that they know what, when and how to treat it. Is that really true? The fact is that there are too many cases when the diagnosis was overlooked [1]. As a matter of fact, it is a common dermatological and surgical problem.

Hidradenitis suppurativa is a chronic, recurrent, inflammatory and painful process in the follicles of hair and near a sweat gland. It is characterized by a thickening of the affected skin together with knots and sinuses and development of ugly scars [2]. Topologically, it primarily affects the skin of the folds i.e. the skin of the armpits, groin, genitals and perianal region. The disease was precisely defined at the Second International HS Research Symposium in San Francisco in March 2009 [3]. The synonyms for the disease are acne inversa, Verneuil's disease and follicular hyperkeratosis [4].

The objective of the study was to show a small series of patients with pictures and definitive results of the surgical procedure. There may have been a possibility of an oversight in making the diagnosis, as well as an underestimation in the treatment. In addition to surgical methods the authors discuss other therapeutic possibilities taken from the available literature.

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Abbreviations
HS – hidradenitis suppurativa
TNF – tumor necrosis factor

Case Reports

In the three-year period between 2011 and 2013, 8 patients diagnosed with hidradenitis suppurativa were operated on at the regional Department of Surgery. All the patients were male. The localization in 7 of the patients was the perianal and gluteal region. One patient had changes both in the groin and pubis. The age of the patients ranged from 30 to 73 years, the average being 42 years. The retrospective analysis of the surgical findings of all 8 patients, and the interview (with 2 of them after a shorter period of time or a year later) provided the data on the treatment methods applied before the surgery, the length of the treatment, the quality of life before the surgery, especially related to the disease, and, finally, the level of satisfaction after the surgery, both in the functional and aesthetic aspect.

The diagnosis was made in all 8 patients by examination **(Figure 1)**. There was a differential-diagnostic dilemma in 2 patients whether a perianal fistula was present, which proved true in one case intraoperatively. The average length of the treatment before the surgery was over 15 years. They were treated with antibiotics, disinfectants and occasional incisions. Before the arrival to the Department of Surgery, *only one patient had an accurately made diagnosis*. Two of them were treated with the diagnosis of fistulous sinus pilonidalis, whereas pyodermia and perianal arborized fistula was set as a diagnosis in 3 and 1 patient, respectively. They all had a chronic inflammation of the affected region with a continuous secernment to a larger or smaller extent.

Before the indication was set, the disease had been treated by different specialists (a gynecologist, a general medicine specialist, a dermatologist, a surgeon, an occupational health specialist) as a typical recurrent skin suppuration. The patients had been treated with various antibiotics, dermatological mixtures, occasional incisions, disinfectants and ointments. During that time, scars and channels (sinuses) had multiplied, leaving ugly skin deformities.

All the patients who were surgically treated were at the 3rd stage, according to Hurley. Seven patients were operated under the general anesthesia by the same team, and one received the peridural anesthesia. A wide surgical excision was applied to all of the patients **(Figure 2)**. The excision to the fascia was performed in 5 cases, the excision near the fascia with leaving a layer of fat tissue was done in 3. The injection of methylene-blue into the sinuses was not done. None of the cases included the covering of the tissue defect with a skin graft. The skin defect was narrowed by rotation flaps and Z plastic in 3 patients **(Figure 3)**. A larger part of the postoperative wound healed per secundam **(Figure 4).**

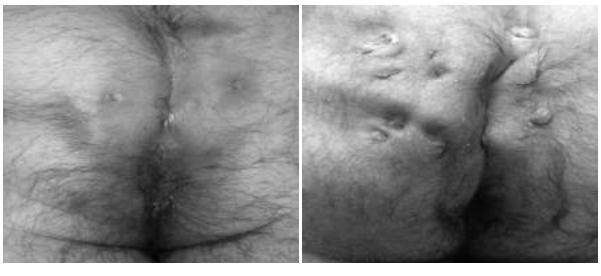

Figure 1 a and b. Hidradenitis suppurativa gr. III
Slika 1 a i b. *Hidradenitis suppurativa gr. III*

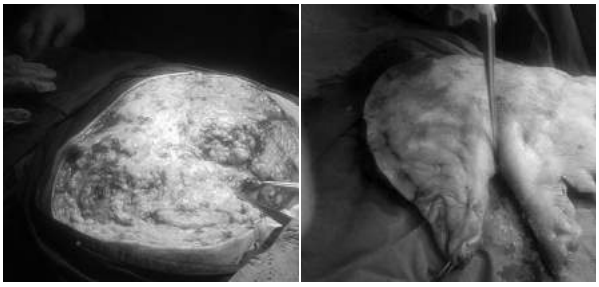

Figure 2 a and b. Wide excision of affected tissues
Slika 2 a i b. *Široka incizija zahvaćenog tkiva*

The patients were interviewed both before and after operations and answered the questions regarding the length of the preoperative treatment and the level of the preoperative dissatisfaction regarding the disease. All the patients had been irritated by the disease manifestation, and two of them claimed it to be a general state of dissatisfaction. One had even been treated for depression.

None of them suffered the HS relapse, except the patient with the perianal fistula (outer opening 10 cm from the anus). The fistula was diagnosed during the operation. All but one patient were operated in the prone position, slightly bent on the operating table. The length of the hospital treatment was between 7 and 14 days or until granulation started to develop, and the total treatment with bandaging in an outpatient department lasted over 2 months, all the way to the complete forming of a stable scar and to a partial epithelization.

During the postoperative interviews, all operated patients showed functional satisfaction, even the one with the fistula recurrence. But the youngest and unmarried patient expressed an aesthetic dissatisfaction. It points to the psycho-social importance of the disease [5].

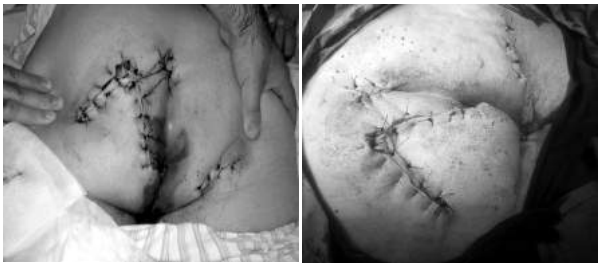

Figure 3 a and b. Primary reconstruction
Slika 3 a i b. *Primarna rekonstrukcija*

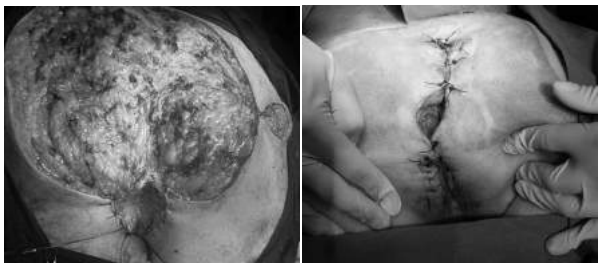

Figure 4 a and b. Secondary stitch of the postoperative wound
Slika 4 a i b. *Sekundarni šav postoperativne rane*

Discussion

No increase in the number of the HS patients has been noticed in the last decades, but it is sure that many papers on the disease have been published in the past 10 years [6]. Although HS is not that rare, both the oversights in making its diagnosis and the underestimation in its treatment are quite frequent. The duration of the disease until the surgery is between 7 and 19 years [3]. As for our patients, the average time elapsed from the onset of symptoms up to the operation was 15 years. The age of all our patients ranged from 15 to 40 years (except the one who was 73), that being the same as in most of the published works. All our patients were male contrary to the statistics found in the literature.

Is it a rare disease or not? By the European criteria, a rare disease is the one with the frequency lower than 5 in 1000 cases or 0.05%, and the American criteria recognize the frequency bellow 0.08 %, i.e. less frequently than 1 in 1250 people [7]. The European literature quotes the disease frequency of 1-4% [7, 8]. Some American sources mention the frequency of only 0.053 %, which is a greatly underestimated prevalence [9]. The most abundant data are given by Naldi L. in his analysis of 9 studies which mention the frequency ranging from 4 in 100 to 1 in 3000. He explains such a discrepancy by methodological differences. Nevertheless, he comes to a *conclusion that the value of 1%* is the most precise for many European countries [10]. Most authors quote 2 to 5 times greater frequency in women and the ages affected by the disease ranging from puberty to 40 years of age [1, 11].

Its pathogenesis is unknown. It is common knowledge that hyperkeratotic production occurs in the hair follicle and leads to the occlusion of the output channel. Thus created cystoid formation grows into a small abscess by the intrafollicular bacteria multiplication. The inner follicular pressure increases and causes the rupture of the cystoid formation and the creation of a tract in the surrounding subcutaneous tissues – fistulation. Healing and recurrence of the inflammation lead to the hardening and thickening of the tissue, as well as to the development of the chronic infection with the skin maceration [12]. But the right question is what initiates these processes. Etiological causes are: genetic factors (family predisposition with the frequency of 30-40% [13]), overweight, smoking, changed cutaneo-immunological response and hormone stimulation. The presence and multiplying of bacteria together with a frequent mechanical skin irritation (friction) bring about the development and aggravation of the disease [14]. We think that is the cause of disease in our patients.

Thorough anamnesis and close examination are all we need to diagnose the disease. The localization and characteristic changes strongly suggest HS. A biopsy may be performed only if a combined disease is suspected [3]. The differential diagnosis takes into consideration infected acne, furunculosis, "cat scratch disease", cellulitis, cutaneous blastomycosis, epidermoid cyst, erysipelas, granuloma inguinale and lymphogranuloma venereum. It may co-exist with the following diseases: perianal fistula, sinus pilonidalis, Crohn's disease, irritable colon syndrome, Down's syndrome, some forms of arthritis, Graves' disease, Hashimoto's thyroiditis and Sjögren's syndrome [12]. HS shares the same immunological etiology with some of these diseases. Certain syndromes, in which HS is a part of the clinical picture, are precisely defined, for example, pyogenic arthritis, pyodermia gangrenosum, acne and hidradenitis suppurativa (PAPASH) – "PASH", Pyogenic Arthritis, Pyoderma gangrenosum and Acne (PAPA) and others have been noted earlier, too. They are believed to be the consequences of gene mutation [15]. One of our patients had the recurrence of perianal fistula. An internal opening may be found by preoperative physical examination in about 75% of the patients and by transanal ultrasound (TUS) in 95%.

Hidradenitis suppurativa can also be the cause of the squamous-cellular carcinoma, especially in the perianal region [16].

According to the morphological criterion set by Hurley in 1989, the HS classification has 3 stages:

1. Isolated knots ("a blind boil")
2. Forming of fibroses and fistulous (sinus) channels not connected into a unique area
3. Confluent fistulae, knots and hypertrophic scars [17].

There is also Sartorius's classification of the HS stages [18]. The treatment mostly depends on the disease stage: mild, medium and heavy forms which correlate with Hurley's morphological picture. Even a few lethal outcomes due to HS have been reported.

Hidradenitis suppurativa was classified into Hurley stage III in our patients. The quality of life is always severely decreased in the HS patients, especially in the stages of the disease which are graver and more spread on the skin. In the most frequently affected age group, the disease can cause both psychological and physical disorder of the patients' sex life. In any case, the social life of the patients is affected, and their professional abilities are diminished. That makes the social support and psychological treatment necessary.

The basic principle in the management of HS is that an early and thorough surgical excision gives the best results. The treatment includes:

– a local treatment: disinfectants, light (photodynamic), laser treatment, local antibiotics and other solutions, which are not used excessively;
– a pharmacological and medicamentous treatment: immunosupressants and immunomodulators, tumor necrosis factor (TNF) alpha inhibitors, antibiotics, oral retinoids and anti-androgens, which are not at our disposal;
– a surgical treatment: "deroofing", wide excision; skin grafts and other esthetic operations as a temporary solution;
– a radiological treatment – an external radiation, not used by us;
– and a psychological treatment.

A radical surgery can be delayed in stage I. As for the later stages, it is best to have a surgical intervention as soon as possible, as our experience shows. Generally, the operation can be delayed during stage I. Instead, it is recommended to shave the region and administer antibiotics according to the antibiogram. Local treatment of rinsing with antibiotic and disinfecting solutions is also recommended. The most common isolated causes of an infection are: Staphilococcus aureus (51%), followed by Escherischia colli, Streptococcus, and anaerobes [15]. Various antibiotics can be administered, such as clindamycin, cephalosporin, tetracycline, rifampicin and others. Photodynamic therapy has been introduced recently, as well as laser therapy for stages I and II [15]. However, neither of these two therapies has been used in our cases. The change of lifestyle in terms of a weight loss and giving up smoking affects the treatment results in some patients. Because of the high percentage of recurrence, the patient should be prepared psychologically for a long-lasting treatment and, finally, a surgical intervention [17].

According to Hurley, the surgical treatment is applied in stage II and III. It can be performed alone or in the combination with a medication therapy.

Available literature data show that the following medications are used in the treatment: immunomodulatory medications (anakinra, cinakinumab, and ustekunimab, which improves the condition in 2/3 of the HS patients and is also used in psoriasis, as well as azathioprine, tacrolimus, thalidomide) [7], anti-inflammatory TNFα-inhibitors (infliximab, etanercept, adalimumab), immunosupressors (prednisone), dapson, antiandrogens and acitretin. They have good effects when the disease is of the autoimmune genesis. Since all our patients were in stage III of the disease, this treatment was not used.

Surgical treatment of our patients always included a wide excision of the change in the gluteal region going to the fascia in 5 cases (one of them with several different excisions of the affected cutaneous and subcutaneous areas) and with the removal of the most part of the subcutaneous, fat tissue except a thin layer above the fascia i.e. "almost to the fascia" in the other 3 cases. Most authors agree that the surgical treatment has no alternative in other types of treatments if we want to come to a definite solution of the problem [19–22]. A dilemma is whether to perform the excision to the fascia or "almost to the fascia" until the granulation begins [23]. At the same time we had in mind the principles of plastic surgery [24].

After the excision of the affected region, the following is possible: 1. leaving the wound to heal per secundam, 2. healing per secundam with a partial closing of some of the portions, 3. delayed grafting with an autologous skin portion in its full thickness [23], and 4. delayed grafting with an artificial graft [25]. The choice depends on the size of the defect, risk of a recurrence, risk of an infection and expected aesthetic result. The healing per secundam and partial narrowing of the excised area by skin portions, Z plastic, etc. have the lowest percentage of recurrence and infection but the longest treatment and poorest aesthetic result [25]. The grafting with skin in its full thickness gives good results, but has a higher percentage of infection [26]. More and more authors advocate the grafting with an artificial skin graft. However, considering the graft thickness and aesthetic aspect, it is not recommended to do the excision to the fascia, but more moderately [27].

We did not perform the surgical procedure of cutting and opening the sinus tracts with the removal of their surface parts ("deroofing") and we think it is a palliative measure with a high percentage of relapsing [28]. It is good for the management with multiple suppurations in an outpatient department. We used simple and multiple incisions as a temporary procedure.

Some time ago, many authors recommended X-rays and laser to be applied before the final operation (neodymium: yttrium-aluminium-garnet laser to be applied for three months or carbon dioxide laser). The logical goal was to "dry" the affected area by the application of the outer radiation in the amount of 3-8 Gy in several fractions. A study performed in 2000 reported radical withdrawal of the changes in 1/3 of 231 cases [22].

Conclusion

Hidradenitis suppurativa is not a rare disease. The choice of the treatment methods is wide. It is sure that keeping records of the patients improves the treatment – as they do in the Scandinavian countries with the so-called HISREG (Clinical Scandinavian Registry for Hidradenitis Suppurativa) method of case registration. According to our experience, surgical treatment is not indicated in the early stages of hidradenitis suppurativa. In the advanced stages of disease, only a surgery can lead to healing. The affected regions are characteristic. The affected region in all our patients was the gluteal region. When scars and sinus fistulae begin to form, an early operation prevents the creation of more serious esthetic consequences. The operative management is a wide excision without compromise and with one of the described ways of the operative wound closing. Recurrences on some parts of the

operated area are possible even after the surgery. In that case, a re-excision is performed. Our patients did not suffer recurrences of the primary disease. The disease may be combined with other diseases such as: fistulae, pilonidal sinus, immunosuppressive states, or within the mentioned syndromes.

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Case report
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ATYPICAL KAWASAKI DISEASE

ATIPIČNA KAVASAKIJEVA BOLEST

Ljiljana RISTOVSKI[1], Olgica MILANKOV[1,2], Melanija VISLAVSKI[1], Radojica SAVIĆ[1,2] and Milena BJELICA[1]

Summary

Introduction. Kawasaki disease is an acute vasculitis which occurs primarily in children under the age of 5. The etiology of the disease is still unknown. Diagnostic criteria for Kawasaki disease are fever and at least four of the five additional clinical signs. Incomplete Kawasaki disease should be taken into consideration in case of all children with unexplained fever for more than 5 days, associated with 2 or 3 of the main clinical findings of Kawasaki disease. The diagnosis of incomplete Kawasaki disease is based on echocardiographic findings indicating the involvement of the coronary arteries. Cardiac complications, mostly coronary artery aneurysm, can occur in 20% to 25% of untreated patients and in 4% of treated patients. **Case Report.** In this report we present a case of atypical Kawasaki disease in a 3.5-month-old infant. As soon as the diagnosis was made, the patient received high doses of intravenous immunoglobulin, with the initial introduction of ibuprofen, then aspirin with a good clinical response. Due to the presence of aneurysm of coronary arteries, further therapy involved aspirin and clopidogrel over the following 3 months, and then only aspirin for 2 years. There was a gradual regression of the changes in the coronary blood vessels to the normalization of the echocardiographic findings after 2 years. **Conclusion.** Kawasaki disease is the second most common vasculitis of childhood, so it should be included in the differential diagnosis for any child with a prolonged unexplained fever. Atypical Kawasaki disease should be taken into consideration in cases when not all clinical criteria are present but coronary abnormalities are documented.

Keywords: Mucocutaneous Lymph Node Syndrome; Infant; Child, Preschool; Signs and Symptoms; Fever of Unknown Origin; Vasculitis; Coronary Vessel Anomalies; Immunoglobulins, Intravenous; Aspirin; Diagnosis; Treatment Outcome

Sažetak

Uvod. Kavasakijeva bolest je akutni vaskulitis koji se prvenstveno javlja kod dece mlađe od 5 godina. Etiologija bolesti je još uvek nepoznata. Dijagnostički kriterijumi za Kavasakijevu bolest su povišena telesna temperatura i najmanje četiri od pet dodatnih kliničkih znakova. O atipičnoj Kavasakijevoj bolesti treba misliti kod sve dece sa neobjašnjivom febrilnošću koja traje duže od 5 dana, koja je praćena sa dva ili tri dodatna klinička znaka. Dijagnoza atipične Kavasakijeve bolesti bazirana je na ehokardiografskom nalazu koji ukazuje na zahvaćenost koronarnih arterija. Srčane komplikacije, najčešće aneurizma koronarnih arterija, sreću se kod oko 20−25% nelečenih pacijenata i kod oko 4% lečenih pacijenata. **Prikaz slučaja.** Opisan je slučaj atipične Kavasakijeve bolesti kod odojčeta uzrasta 3,5 meseca. Po postavljanju dijagnoze dete je primilo visoke doze intravenskih imunoglobulina, sa inicijalnim uvođenjem ibuprofena, potom i aspirina, uz dobar klinički odgovor. S obzirom na prisustvo aneurizme koronarnih arterija, dalja terapija je nastavljena aspirinom i klopidogrelom naredna tri meseca, potom samo aspirinom tokom dve godine. Došlo je do postepene regresije promena na koronarnim krvnim sudovima, sve do normalizacije ehokardiografskog nalaza nakon dve godine. **Zaključak.** Kavasakijeva bolest je drugi po učestalosti vaskulitis u dečjem uzrastu, te treba biti uključena u diferencijalnu dijagnozu kod svakog deteta sa prolongiranom febrilnošću nepoznatog uzroka. U slučajevima kada nisu prisutni svi klinički kriterijumi bolesti, ali je utvrđeno postojanje promena na koronarnim krvnim sudovima, potrebno je razmotriti atipičnu Kavasakijevu bolest.

Ključne reči: Kawasakijeva bolest; Odojče; Predškolsko dete; Znaci i simptomi; Groznica nepoznatog uzroka; Vaskulitis; Anomalije koronarnih krvnih sudova; Intravenski imunoglobulini; Aspirin; Dijagnoza; Ishod lečenja

Introduction

Kawasaki disease (KD) was first reported by Tomisaku Kawasaki in a four-year-old child (Red Cross Hospital in Tokyo, Japan, January 1961) [1]. This disease is an acute vasculitis in children with the mortality rate of 0.1-2%. The incidence among Asian children is significantly higher than in children from other regions, 134-135 per 100,000 children under 5 years of age, but this disease occurs throughout the world and in all ethnic groups [2–4]. KD occurs primarily in young children, with 80% of patients under the age of 4, and with the peak incidence occurring at 9 to 11 months of age. The illness is extremely rare in infants younger than 3 months of age, while the youngest reported patient in the literature is a 2-week-old neonate [5]. The disease is more common in male children, the ratio between boys and girls being 1.7:1 [1].

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Abbreviations:

KD – Kawasaki disease
IVIG – intravenous immunoglobulin
MI – myocardial ischemia
ESR – erythrocyte sedimentation rate
CRP – C-reactive protein
HDL – high-density lipoprotein
GGT – gamma-glutamyl transferase

The etiology of the disease is unknown, but clinical and epidemiological presentation suggests an infectious origin. However, a particular virus or bacteria has not been implicated. There are circumstantial data supporting the role of certain bacterial toxins (e.g. staphylococcal toxic shock toxin, streptococcal erythrogenic toxin), viruses (Epstein-Barr virus, parvovirus, human immunodeficiency virus (HIV)-2) or ubiquitous microorganisms that cause clinically manifested disease in genetically predisposed individuals. Considering the fact that the disease is more common in children of Asian origin, it is likely that genetic factors play a role as well [2, 6, 7]. This theory is corroborated by the fact that the disease is more common in twins and siblings of affected patients than in general population, and is more common in children whose parents had the illness in their childhood [6].

Kawasaki disease consists of generalized systemic vasculitis, affecting predominantly medium-sized vessels [1]. Coronary arteries are most frequently affected, but the process may occur in other extraparenchymal muscular arteries such as celiac, mesenteric, femoral, iliac, renal, axillary and brachial artery [6].

The diagnosis is based on the recognition of characteristic clinical signs [1]. It is important to realize that often not all of the symptoms are present at the same time, so it is necessary to repeat clinical examinations in order to make the diagnosis [7]. Diagnostic criteria for KD are fever and at least four of the five additional clinical signs. Fever within KD can last from 5 to 25 days, usually reaches 40°C and is unresponsive to antibiotics and minimally responsive to antipyretics. Other clinical signs are: 1. bilateral bulbar conjunctival injections not accompanied by suppuration, usually without involvement of the limbus, often accompanied by photophobia; 2. changes in the mucosa of the oropharynx (bright red, swollen lips with vertical cracking and bleeding, red oropharynx, strawberry tongue); 3. changes in the peripheral extremities (erythema of the palms and soles, which is often accompanied by painful, brawny edema of the dorsa of the hands or the feet in the acute phase, followed by desquamation of the fingers which begins at the periungual region); 4. polymorphous rash, usually diffuse maculopapular, although it can be scarlatiniform, multiform, urticarial, rarely micropustular, but never bullous or vesicular; 5. cervical lymphadenophathy (more than 1 lymph node exceeding 1.5 cm in diameter), without suppuration, usually unilateral, with involvement of the front cervical lymph nodes [1, 6, 7].

The least common clinical sign is cervical lymphadenopathy, which is present in only about 50% of children with KD and significantly more often in older children [8]. Younger children with KD tend to be more irritable. Although not included in the diagnostic criteria, gastrointestinal symptoms such as nausea, vomiting and diarrhoea, as well as arthritis and arthralgia, are common in children with KD [9]. In addition, other clinical manifestations may be present in some cases such as aseptic meningitis, facial palsy, ataxia, encephalopathy, hemiplegia, cerebrovascular accident, sensorineural hearing loss, pleural effusion, pulmonary infiltrates, otitis media, acute renal failure, abdominal pain, hepatitis, gallbladder hydrops, pancreatitis, jaundice, swelling of the testicles, urethritis and, very rarely, rhabdomyolysis and hemophagocytic syndrome [1, 3, 4, 6].

Incomplete or atypical KD should be taken into consideration in case of all children with unexplained fever for more than 5 days, associated with 2 or 3 of the main clinical findings of KD [1]. The diagnosis of incomplete KD is based on echocardiographic findings indicating the involvement of the coronary arteries [1, 6]. Ten percent of children with aneurismal changes in coronary vessels fall into this category. Incomplete clinical presentation of KD is very common in infants, especially under the age of 6 months [7]. Two most commonly absent symptoms in atypical cases are cervical lymphadenopathy and polymorphous rash while mucosal changes are nearly always found [3].

Kawasaki disease is usually self-limiting, having a triphasic course, with an average duration of symptoms of approximately 12 days [3, 7]. The acute phase begins with fever, conjunctivitis, oral changes, lymphadenopathy and rash and lasts for almost one to two weeks. The subacute phase is characterized by desquamation of the hands and feet and conjunctivitis may also persist during this phase. The convalescent phase starts when all the clinical signs have resolved and ends when the laboratory abnormalities have returned to normal, usually four to six weeks after the onset of disease [3].

The diagnosis of KD is based on clinical characteristics; there are no confirmatory laboratory tests. However, certain laboratory tests can be used to support the diagnosis, such as elevated erythrocyte sedimentation rate (ESR) (≥40 mm/h) or elevated C-reactive protein level (CRP) (≥3 mg/l), leukocytosis (≥15000/μL) with predominance of granulocytes, normochromic and normocytic anemia, sterile pyuria (≥ 10 leukocytes in the sediment, although suprapubic urine generally does not show piura, which suggests urethritis) and proteinuria. It is expected to find a moderate increase in the levels of transaminases, especially serum alanine aminotransferase >50 U/L (can be found in about 30% of patients due to congestion of the liver), gamma glutamyl transferase (GGT) increase (in almost 70% of patients), mild hyperbilirubinemia (in about 10% of patients), hypoalbuminemia, thrombocytosis ≥450000/mm3 (an increase in the platelet count is recorded in the second week of illness with a peak in the third week; thrombocytopenia is very rare and is a risk factor for coronary aneurysms), abnormal serum lipid levels, in-

cluding elevated triglyceride levels and low-density lipoprotein (LDL) levels and decreased high-density lipoprotein (HDL) levels, hyponatremia (<135 mEq/L, which is associated with an increased risk of coronary artery aneurysms) and elevated serum IgE levels. Pleocytosis with predominance of mononuclear cells can be detected in the cerebrospinal fluid, as well as the inflammatory cells in the synovial fluid [1, 6, 7]. Clinical experience suggests that KD is unlikely if acute-phase inflammatory reactants and platelet count are within normal values after seven days of illness [6].

Kawasaki disease, which was initially thought to be a benign, self-limiting febrile illness, is now known to be associated with sudden death in about one percent of the affected children due to acute coronary vasculitis, which leads to thrombus formation in the affected vessels and myocardial infarction [9]. Cardiac complications can occur in 20% to 25% of untreated patients and in 4% of treated patients. The most common cardiac complication seen in KD is coronary artery aneurysm [7]. Changes in blood vessels usually occur 10 days to 4 weeks after the onset of symptoms, but they may occur earlier, while their presence for more than 5 weeks after the onset of fever is uncommon [7, 9]. They tend to resolve in 50% of patients within 5 to 18 months. Expeditious diagnosis is crucial, because treatment with immunoglobulin within the first 10 days of illness significantly reduces the incidence of coronary artery aneurysms [7]. For this reason, it is recommended to perform at least three echocardiograms in children with KD in the first 6 weeks of the disease [10].

Since the etiology is unknown, pharmacological therapy is non-specific and directed towards modulation of the inflammatory response and inhibition of platelet activation with the aim of preventing coronary artery aneurysms [11]. Treatment should begin with intravenous immunoglobulin (IVIG) and high doses of aspirin as soon as the diagnosis is made. The recommended dose of IVIG is 2 g/kg given as a one-time infusion during an 8- to 12-hour period. It is recommended to start this therapy within the first 10 days of the illness. The exact mechanism of IVIG's action is unknown, but it appears to have generalized anti-inflammatory activity [7]. In addition to prevention of coronary artery aneurysms, normalization of lipid profiles and improvement of cardiac contractility seem to be other effects. The patient should also be given aspirin for its antiinflammatory and antiplatelet activity. In addition to aspirin, other antiplatelet drugs can be used such as clopidogrel and inhibitors of the platelet glycoprotein IIb/IIIa receptor [1, 11]. For treatment of IVIG-resistant patients, a variety of therapies has been tried including repeated dose of IVIG, high-dose pulse methylprednisolone, cyclophosphamide or methotrexate, but there is no established guideline for the choice of treatment. One possibility is the use of infliximab, a monoclonal antibody against tumor necrosis factor alpha (TNF-alpha), as well as plasmapheresis [12].

The overall prognosis for patients with KD depends on the severity of coronary artery involvement as a risk factor for myocardial ischemia (MI). Patients with aneurysms larger than 8 mm are at the highest risk for MI. Aneurysms that are 8 mm or smaller tend to regress over time, and those that are 6 mm or smaller tend to resolve completely. Patients without any cardiovascular abnormalities tend to do well, and are generally asymptomatic at their long-term follow-up examination [7].

Case Report

The patient is a 3.5-month-old female infant, who developed the symptoms of fever (39.2 ^{o}C axillary), cough and diarrhea, followed by redness of the skin on the trunk next day. Ambulatory oral antibiotic therapy (amoxicillin) was introduced. However, the child was hospitalized the same day because of the recurrence of fever. On admission to hospital, the child had clinical signs of disease in the form of rose-colored exanthema on the trunk, dry and intensely red lips, extreme hyperemia of the mucosa of the oropharynx, bilateral suboccipital and submandibular lymph nodes palpable to 0.5 cm. The laboratory analysis showed a high level of CRP (96 mg/l), elevated ESR (48 mm/1h), leukocytes within normal ranges, prolonged activated partial thromboplastin time (APTT) (37.7 sec), elevated level of GGT (2.04 ukat/l) and increased titters of specific IgM antibodies for Adenovirus and Epstein Barr virus. Antinuclear, antimitochondrial, antiparietal, anti-smooth muscle antibodies, serum immunoglobulin, C3 and C4 complement components were within normal ranges. Urine analysis showed proteinuria and sterile pyuria (25-30 leukocytes in the sediment, uricult negative). Bacteriological culture of blood, cerebrospinal fluid and stool were negative. Staphylococcus aureus was isolated from the nasal swab, while the throat swab showed Candida albicans in large numbers. Ultrasound of the neck pointed to the enlargement of lymph nodes along the sternocleidomastoid muscle, of approximately 12.5 mm in diameter. Echocardiography detected a previously diagnosed heart defect (atrial septal defect and stenosis arteriae pulmonalis) with suspected aberrant coronary blood vessel. The child was examined by an otorhinolaryngologist three times to exclude focal point in the otorhinolaryngeal region.

As soon as the patient was hospitalized, parenteral antibiotic therapy (ceftazidime) was introduced with antipyretic for a suspected systemic infection. Despite the applied therapy, the child had daily 4 to 5 episodes of high fever (39.4 °C). Initially present macular rash decreased gradually, while the redness of the lips and buccal mucosa maintained, with the appearance of strawberry tongue and conjunctival injection. In the further course, diarrhea started with 5-7 watery stools. On the 7th day of hospitalization another parenteral antibiotic (amikacin) was introduced. Since the febricity continued with persistent clinical findings and an increase in the level of acute-phase inflammatory reactants (CRP 96 mg/l, ESR

78 mm/1h, procalcitonin 0.09 ng/ml, leukocytes 19,82 x 10^9/l, platelets 806 x 10^{12}/l), the diagnosis of atypical KD was made after other differential diagnostic possibilities had been excluded. On the 13th day of hospitalization the patient received high doses of IVIG (a total of 11 g for 4 days, about 1.8 g/kg), with initial introduction of ibuprofen 30 mg/kg/day, then aspirin 50 mg/kg/day. The child became subfebrile after the first dose of IVIG and the following day the child was afebrile. All previously described clinical signs of disease withdrew and the laboratory parameters returned to the normal values. During the hospitalization, the child was repeatedly examined by a cardiologist with echocardiographic evaluation at the beginning and at the end of treatment. The initial finding, as previously described, spoke in favor of aberrant coronary artery, while the subsequent ultrasound monitoring, after completion of IVIG therapy, detected suspected dilatation of this vessel. Because of the need for sophisticated cardiac diagnostic and monitoring procedures and the possible development of complications, the child was transferred to the Institute for Health Protection of Mother and Child in Belgrade on the 17th day of hospitalization. During hospitalization in that institution, the aneurysm of both coronary arteries was diagnosed with the presence of thrombus, so further therapy involved continued administration of aspirin (5 mg/kg/day) and clopidogrel (0.13 mg/kg/day) over the following 3 months, and after that period only aspirin (8 mg/kg/day) for 2 years. In the further course of treatment there was a gradual regression of the changes in coronary blood vessels, to the normalization of the echocardiographic findings after 2 years.

Discussion

Kawaski disease is a multisystem vasculitis mainly affecting medium-sized blood vessels. It is the second most common cause of vasculitis after Henoch Scholein Purpura (HSP) in children [13]. In a study sample of 1,374 patients with KD, only 61 patients (4%) were under 6 months of age at diagnosis, while 114 patients (8%) were between the ages of 6 months and a year [14]. Therefore, our patient, aged 3.5 months, belongs to the age group where the occurrence of KD can be expected, but it is a rarity. This disease more frequently occurs is boys [1]. In contrast, in our case it was a female infant. The etiology of the disease remains unknown. It is considered that an autoimmune and genetic component may have an impact on the occurrence of the disease. A possible infectious agent is often mentioned as well [13, 15]. In our case, all bacteriological cultures taken were negative, nasal swab detected Staphylococcus aureus, while the specific IgM antibodies for some virus were elevated, indicating the actual viral infection.

It is important to keep in mind that about 20% of fevers in childhood have no apparent cause. A significant number of these children may have a serious bacterial infection. Whenever the fever of unknown cause is present in children 0-36 months of age, we should closely monitor these children and thoroughly seek for the cause of fever [16]. Diagnosis of KD is based on clinical criteria. The most important and the only criterion which is always present is prolonged fever, lasting longer than 5 days. It cannot be reduced by antibiotic therapy and is relatively resistant to antipyretics [1]. This criterion is met in the case of our patient, in whom fever persisted despite the applied dual parenteral antibiotic therapy and antipyretics. Other criteria are the presence of bilateral conjunctivitis, changes of the oropharyngeal musoca, and changes in the periphery of the extremities, cervical lymphadenopathy and rashes on the skin [7]. Our patient had three of the five criteria, which is a characteristic of atypical manifestation of KD. Exanthema on the trunk and severe oropharyngeal mucosal hyperaemia were present at the very beginning of the disease, while in the further course bulbar hyperaemia appeared.

In addition to these clinical signs of disease, which are important criteria for the diagnosis, KD can be characterized by the presence of clinical manifestations of other organ systems [7]. Our patient had diarrhea as well. According to the literature, the disease often manifests with less than 4 characteristic criteria in children under 6 months and over 5 years of age, which is beyond the typical age for KD appearance. These children are also at greater risk for making a diagnosis after 12 days from the onset of the disease [14]. All of this was confirmed in the case of our patient.

As far as laboratory analysis is concerned, no single finding is crucial for the diagnosis of KD. However, associated pathological values of certain laboratory parameters can help us to make the diagnosis. In case of KD and related laboratory findings, we expect an increase in the level of acute-phase inflammatory reactants, anemia, thrombocytosis, hypoalbuminemia, hyponatremia, hypertriglyceridemia, a decreased level of HDL with an increased level of transaminases [1, 6, 7]. In our patient, the laboratory analysis revealed an increase in CRP, fibrinogen and procalcitonin level, leukocytosis, thrombocytosis and an increased level of GGT. Urine analyses of patient with KD often show sterile pyuria and proteinuria [6], as observed in our case. Other laboratory tests were within normal ranges.

When KD is diagnosed on the basis of clinical manifestations and laboratory criteria, the therapy should be immediately introduced in order to prevent possible complications [10]. The aim of the therapy in the acute phase is to reduce inflammation in the walls of the coronary arteries and to prevent coronary thrombosis [6]. Treatment using a high dose of IVIG with aspirin, based on randomized controlled trials and meta-analyzes, clearly reduces the risk of occurrence of aneurysm of the coronary arteries. Two g/kg of IVIG is the optimal dose, usually given as a single infusion and this regimen is considered to have a greater therapeutic effect in prevention of aneurysm

comparing to the 4-day regimen (400 mg/kg/day for four consecutive days). Currently, aspirin at a dose of 30–50 mg/kg/day is recommended during the acute phase of the illness. It has not been proved that treatment with higher doses of aspirin is more effective in reducing the incidence of complications. Lower doses are better tolerated in terms of gastrointestinal and other side effects [10].

In case of our patient, the diagnosis was not made at the onset of the disease due to the atypical clinical presentation, and the treatment started at a later phase of the disease, on the 13th day of hospitalization. Our patient had been receiving IVIG for four days in a total dose of 1.8 g/kg with aspirin in a dose of 50 mg/kg/day and the clinical improvement was observed immediately after the introduction of therapy with IVIG and aspirin, and the laboratory parameters returned to the normal values. Cardiac complications can occur in 20% to 25% of untreated patients and in 4% of treated ones. Patients under 1 year of age are more likely to have coronary artery abnormalities [7, 14]. In spite of the applied therapy, some complications appeared in our patient. Echocardiographic monitoring revealed aneurysms of the coronary arteries with the subsequent occurrence of thrombosis on the 13th day of treatment. Long-term therapy for patients who develop coronary artery aneurysms is aimed at preventing myocardial ischemia and myocardial infarction [6]. If aneurysms persist, low doses of aspirin are recommended. Clopidogrel is an alternative therapy that may be taken into consideration [10]. In our case, both aspirin and clopidogrel were initially introduced due to the persistence of aneurysms and in the later course only aspirin was continued leading to gradual regression of the mentioned complications after 2 years.

Conclusion

Kawasaki disease is the second most common vasculitis of childhood, so it should be included in the differential diagnosis for any child with a prolonged unexplained fever. Atypical Kawasaki disease should be taken into consideration in cases when not all clinical criteria are present but coronary abnormalities are documented. Making the early diagnosis is the main challenge for the physicians because treatment in the first ten days modifies the prognosis of the disease. As soon as the diagnosis is made, the therapy should start and the patient should be carefully re-evaluated for possible development of complications.

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HISTOPATHOLOGICAL ANALYSIS AND SURGICAL TREATMENT OF BREAST CANCER – OUR EXPERIENCE

HISTOPATOLOŠKA ANALIZA I HIRURŠKI TRETMAN RAKA DOJKE − NAŠE ISKUSTVO

Džemail S. DETANAC[1], Dženana A. DETANAC[1], Avdo ĆERANIĆ[1] and Merima A. ĆERANIĆ[2]

Summary

Introduction. The aim of this study was to show the descriptive and histopathological analysis and applied surgical technique with early and late post-operative complications in patients with breast cancer who were hospitalized and treated at the General Hospital in Novi Pazar during the period 2009-2011. **Material and Methods.** During the period from 2009 to 2011, 59 patients were operated for breast cancer at the General Hospital in Novi Pazar. The study included the size and type of the tumor, disease stage, surgical techniques and complications, the age of the patients at the moment of surgery and its correlation with the number of metastatic lymph nodes in the axilla and the tumor size, as well as the correlation of the tumor size with the number of metastases in the axillary lymph nodes. **Results.** The difference in the tumor size in relation to the age among the women under 50 and over 50 years of age was not statistically significant ($T = -1.203$, $p>0.05$). There was no statistically significant difference between the number of positive lymph nodes in the women under and over 50 years of age (Mann-Whitney U test, $p>0.05$). A significant positive correlation between the tumor size and the number of positive axillary lymph nodes was found ($\rho= 0.308$, $p<0.05$). A significant positive correlation of the patient's age and breast cancer stage was also confirmed with nonparametric variance analysis by Spearman's Rho ($\rho= 0.337$, $p<0.05$). **Conclusion**. The majority of women from this study sample were with Stage II of breast cancer, which points out the necessity for better prevention and education of women in order to improve early diagnosis of breast cancer. The number of positive axillary lymph nodes appears to be an important prognostic factor and a significant positive correlation between the tumor size and the number of positive axillary lymph nodes has been found.

Key words: Breast Neoplasms; Surgical Procedures, Operative; Morphological and Microscopic Findings; Postoperative Complications; Lymph Nodes; Early Diagnosis of Cancer; Neoplasm Staging; Age Factors

Sažetak

Uvod. Deskriptivna i histopatološka analiza bila je cilj studije, kao i analiza primenjenih hirurških tehnika, kao i ranih i kasnih postoperativnih komplikacija kod pacijentkinja operisanih od karcinoma dojke u toku 2009-2001. godine, u Opštoj bolnici Novi Pazar. **Materijal i metode.** Studijom je obuhvaćeno 59 pacijentkinja operisanih tokom perioda 2009-2011. godine. Veličina i tip tumora, stadijum bolesti, hirurške tehnike i komplikacije, godine starosti u vreme operacije, njihova korelacija sa veličinom tumora i brojem pozitivnih aksilarnih limfnih nodusa, kao i korelacija veličine tumora i broja pozitivnih limfnih nodusa su ispitani. **Rezultati.** U našem uzorku ne postoji statistički značajna razlika u veličini tumora među grupama pacijentkinja mlađih i starijih od 50 godina ($T = -1.203$, $p > 0,05$). Ne postoji statistički značajna razlika u broju pozitivnih limfnih aksilarnih nodusa među grupama pacijentkinja mlađih i starijih od 50 godina (Man-Vitnejev U-test, $p > 0,05$). Potvrđena je značajna pozitivna korelacija između veličine tumora i broja pozitivnih limfnih nodusa ($\rho = 0,308$, $p < 0,05$), kao i značajna pozitivna korelacija između starosti pacijentkinja i stadijuma tumora ($\rho = 0,337$, $p < 0,05$). **Zaključak.** Kod najvećeg broja pacijentkinja u našem uzorku dijagnostikovan je tumor u II stadijumu, što ističe značaj odgovarajuće prevencije i edukacije žena kako bi se unapredila rana dijagnostika karcinoma dojke. Pozitivni aksilarni limfni nodusi su značajan prognostički faktor. Nađena je signifikantna pozitivna korelacija između veličine tumora i broja pozitivnih aksilarnih limfnih čvorova.

Ključne reči: Karcinomi dojke; Operativne hirurške procedure; Patohistološki nalaz; Postoperativne komplikacije; Limfni čvorovi; Rana dijagnoza karcinoma; Klasifikacija karcinoma; Uzrast

Introduction

With their incidence and high mortality, malignant diseases are at the top of epidemiological studies [1].

Breast cancer is the most common malignancy among women in both developed and developing countries, with 1.38 million of new cases diagnosed in the world in 2008 [2, 3].

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About 430,000 new cases of breast cancers are diagnosed in Europe every year, while in the United States Of America this number is around 250,000 [4, 5]. The incidence is now higher in more developed countries; however, an increase in the incidence of this disease is expected in countries in transition in the future [6].

In our country, breast cancer is diagnosed in up to 4,000 women annually and it results in 1,600 deaths, which accounts for 18% of cancer mortality in general.

The average standardized incidence rate of breast cancer in Central Serbia in the period from 1999-2009 was 60.8/100,000, and the mortality rate was 20. 2/100,000 [7].

The incidence of most malignant diseases grows with the age, and later, older age is one of the risk factors for breast cancer [8] even though today breast cancer often occurs before the age of 30, which used to be very rare. Risk factors are often intertwined and it is difficult to isolate the specific role of each. Most patients are with a history of un- known risk factors [9].

The aim of this paper was to show the descriptive and histopathological analysis and the applied surgical technique with early and late post-operative complications in patients with breast cancer who were hospitalized and treated at the General Hospital in Novi Pazar.

Material and Methods

The analysis included 59 patients operated for malignant breast tumor at the General Hospital in Novi Pazar during the period from 2009 to 2011. Data from medical records, operation and histopathological reports were reviewed.

Histopathological analysis was performed at the Department of Pathology and Forensic Medicine of the General Hospital Novi Pazar in order to examine the size and type of the tumor, disease stage, surgical techniques and complications, the age of the patients at the moment of the surgery and its correlation with the number of metastatic lymph nodes in the axilla and the tumor size, as well as the correlation of the tumor size with the number of metastases in the axillary lymph nodes.

The study sample did not include patients who had been operated for benign breast tumor.

The descriptive and retrospective data analysis was done based on the clinical examination, pathohistological analysis of tumors and performed surgical techniques.

The findings were summarized by means of the methods of descriptive statistics. The statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The data were processed by using the Mann-Whitney U-test or the T- test, depending on the number and distribution of the compared groups. Spearman's Rho was calculated as a non-

Table 1. Characteristics of the disease and surgical techniques
Tabela 1. *Karakteristike bolesti i hirurške tehnike*

Characteristic of disease and types of surgery *Karakteristike bolesti i tip hirurške intervencije*	Total No. of patients (N = 59) No. (%) *Ukupan broj pacijenata (N = 59) broj (%)*
Stage of disease/*Stadijum bolesti*	
O	4 (6.78)
I	9 (15.25)
IIA	22 (37.29)
IIB	7 (11.86)
IIIA	17 (28.82)
IIIB	0
IIIC	0
IV	0
Metastases in lymph nodes/*Metastaze u limfnim čvorovima*	
Yes/*Da*	32 (54.24)
No/*Ne*	27 (45.76)
Tumor size/*Veličina tumora*	
<2 cm	28 (47.46)
2-5 cm	26 (44.07)
>5 cm	5 (8.47)
Type of surgical technique/*Vrsta hirurške intervencije*	
MRM	19 (32.2)
Quadrantectomy/*Kvadrantektomija*	38 (64.4)
Tumorectomy/*Tumorektomija*	2 (3.4)

*MRM- modified radical mastectomy/*Modifikovana radikalna mastektomija*

Table 2. Postoperative complications
Tabela 2. *Postoperativne komplikacije*

Postoperative complications/*Postoperativne komplikacije*	n	%
Early/*Rane*		
Lymphorrhea/*Limforeja*	1	1.69
Wound infection/*Infekcije rane*	1	1.69
Late/*Kasne*		
Lymphedema/*Limfedem*	2	3.39
Relapse/*Recidiv*	1	1.69

parametric correlation coefficient in the clinical outcome between the individual markers. P<0.05 was considered statistically significant.

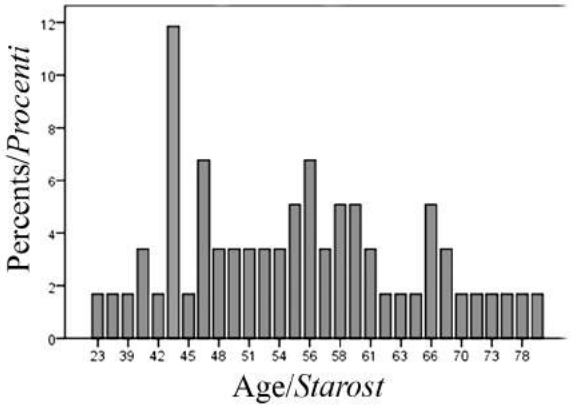
Results

During the period from 2009 to 2011, 59 patients were operated for breast cancer at the General Hospital in Novi Pazar.

The age structure is presented in **Graph 1.** The youngest patient was 23 and the oldest was 79 years old. The average age of the patients at the moment of surgery was 54.37 years. Most of patients (11.9%) were at the age of 43, 37.4% were younger than 50, 5.1% were younger than 40, while 32.3% of women were between 40-49 years of age.

All patients were operated according to decision made by the Consulting team. The preoperative diagnostic preparations included clinical examination, laboratory analyses, radiological examinations (ultrasound, radiography, mammography, computed tomography). All operations were performed under the general anesthesia. At the time of surgery no patient had systemic manifestation of distant metastases.

In our case, most surgical techniques were breast-conserving. Quadrantectomy was performed in 64.4% and tumorectomy in 3.4% of patients. Modified radical mastectomy (Madden technique) was performed in 19 patients (32.2%) **(Table 1).**



Graph 1. Age structure of the patients at the moment of surgery
Grafikon 1. *Starosna struktura pacijentkinja u trenutku operacije*

No intra-operative complications were noted. Early complications developed as lymphorrhea in 1.69% and wound infection in 1.69% of patients. Lymphedema was observed in 2 patients (3.39%) as a late postoperative complication. One patient refused postoperative chemotherapy and she had a relapse after 6 months. The results are shown in **Table 2.**

According to the pathology report, 4 patients (6.78%) had *in situ* stage (stage 0) of the breast cancer, 9 (15.25%) had stage I, 30 (49,14%) patients had stage II, and 17 (28.82%) patients had stage IIIA of the breast cancer. There was no patients with stage IIIC and stage IV **(Table 1).**

Metastases in axillary lymph nodes were found in 32 patients (54.24%) **(Table 1)**.

The size of the primary tumor determined on the basis of the largest diameter measured during the histopathological analysis was found to be less than or equal to 2 cm in 28 (47.46%) patients, between 2-5 cm in 26 patients (44.07%) and larger than 5 cm in the largest diameter in 5 (8.47%) patients **(Table 1)**.

The smallest recorded tumor size was 6 mm, while the largest tumor diameter was 60 mm. The average size of the tumor was 26.49 ± 14.556 mm. The difference in tumor size in relation to the age, among women younger than 50 and those older than 50 years was not statistically significant (T = -1.203, p> 0.05).

The largest number of positive lymph nodes in the axilla found in one patient was 10. The mean number of positive lymph nodes in the axilla was 2.24 ± 2.602. By analyzing the number of positive nodes in relation to the age, no statistically significant difference was observed between the number of positive lymph nodes in women younger and older than 50 years (Mann-Whitney U test, p> 0.05).

Nonparametric variance analysis by Spearman's Rho (ρ= 0.308, p<0.05) revealed a significant positive correlation between the tumor size and the number of positive axillary lymph nodes, meaning the larger the tumor the higher was the number of metastatic lymph nodes in the axilla. A significant positive correlation of the patient's age and the breast cancer stage was also confirmed with nonparametric variance analysis by Spearman's Rho (ρ= 0.337, p<0.05), which means that the breast cancer was more often diagnosed at a higher stage in elderly patients in our study.

Rapid diagnosis during the surgery was performed in all patients. The interpretations were as

Table 3. Histological types of breast cancer
Tabela 3. *Histopatološki tipovi tumora dojke*

	N	%
Ductal carcinoma/*Duktalni karcinom*	37	62.7
Lobular carcinoma/*Lobularni karcinom*	11	18.6
Mucinous carcinoma/*Mucinozni karcinom*	1	1.7
Papillar carcinoma/*Papilarni karcinom*	2	3.4
Ductal carcinoma in situ/*Duktalni karcinom in situ*	4	6.8
Undifferentiated carcinoma/*Nediferentovani karcinom*	1	1.7
Carcinosarcoma/*Karcinosarkom*	2	3.4
Mb Paget/*Padžetova bolest*	1	1.7
Total/*Ukupno*	59	100

follows: malignant lesions in 52 cases (88.13%), which was also confirmed by the definite histological analyses; benign lesions in 1 case (1.7%), but the definite histological analyses showed that it was a malignant lesion. Definitive histological diagnosis was delayed in 6 cases (10.17%) which was later confirmed to be a malignant tumor.

In our sample, the most common histological type of the tumor was ductal carcinoma in 62.7% of patients, while a lobular carcinoma was on the second place, being present in 11 cases (18.6%). The results are shown in **Table 3**.

Discussion

In recent years, there has been a remarkable progress in the diagnosis and treatment of breast diseases, especially breast cancer [10].

If untreated, breast cancer has the fatal outcome. Death is inevitable if patients with breast cancer refuse any treatment. Spontaneous evolution and the length of illness vary, and most often, if not treated, the disease ends in death within three years, but in a small percentage, 1.4%, it can last for more than a decade [1].

Breast cancer is a heterogeneous disease with varied morphological appearances, molecular features, behavior and response to therapy. This tumor continues to remain the most lethal malignancy in women throughout the world. The incidence rates of breast cancer vary worldwide. The highest rate has been reported in the northern America, and the Western Europe [11–13] whereas it is very low in most of the Asian countries [14].

The incidence rates are high in developed regions of the world (except Japan) and low in most of the developing regions [6].

The incidence of most malignant disease increases with the age, and older age is a risk factor for breast cancer. The incidence of breast cancer increases with the age, getting twice higher with every 10 years until the menopause, after which the rate of growth slows down considerably [8, 15].

The most commonly affected women are over 50 years of age [9]. Before the age of 35, breast cancer is rare. Approximately 7% of all breast cancers are diagnosed in women under 40 years of age and less than 4% of women are under 35 years of age. The prognosis tends to be negative when the disease is diagnosed at young age [16, 17].

The average age of patients at the time of operation was 54.37 years in our sample, while the percentage of women over 50 years of age was 63.6%, which correlates with the literature data and 5.1% of patients were under 40 years of age, which also correlates with the results of studies done by other authors.

Axillary lymph node status is the most important prognostic factor. Breast cancer that has spread to the lymph nodes (positive lymph nodes) has a higher risk of recurring and a less favorable prognosis than breast cancer that has not spread to the lymph nodes (negative lymph nodes). The number of positive lymph nodes is also an important prognostic factor. The five-year survival, regardless of the size of the primary tumor, significantly decreases with the increase in the positive lymph node number. So, the five-year survival is 70% if the number of positive lymph nodes is 3, it is 50% if the number of positive lymph nodes is 7-12, and only 28.4% if the number of positive lympmh nodes is 13 and more [18].

In our sample, the average number of positive axillary lymph nodes was 2.24 ± 2.602. Due to the short time period of the study there was no possibility to determine the five-year survival.

Many authors suggest that the number of positive axillary lymph nodes increases with the tumor size in their research [19–21].

In our analysis, it was determined that the correlation between the tumor size and the number of positive axillary lymph nodes was medium strong, which coincides with the results in the literature.

In countries where the screening program for early detection of breast cancer is implemented, there is a trend of detection of tumors at early stages. In countries where there is no screening, tumors are detected at later stages.

Early diagnosis can lead to a dramatic reduction in the size of tumor, better prognostic features, more conservative surgery and improvement of survival [22–24].

Invasive ductal carcinoma accounts for 50-60% of all breast cancer. Many authors in their studies pre-

sented data that this is the most common malignant tumor [25–28]. Similar to other studies, our study found that ductal carcinoma, accounting for 62.7% of the all cases, is the most frequent type of breast cancer. Lobular carcinoma takes the second place with 18.6%.

The surgeon has a fundamental role in diagnosis and treatment of breast cancer, which must be multidisciplinary. The main objectives are early diagnosis and radical treatment in order to cure the patient, or to prolong their life and improve its quality [9]. Breast cancer management involves either modified radical mastectomy (MRM) or breast conservation surgery (BCS) as the primary treatment modality followed by adjuvant treatments based on pathological characteristics [29, 30].

In this regard, breast conservation surgery was performed in 67.8% of cases of our study sample, and modified radical mastectomy was performed in 32.2%.

Conclusion

The fact that the majority of women in our study sample had breast cancer stage II emphasizes the necessity for better prevention and education of women in order to improve early diagnosis of breast cancer. The number of positive axillary lymph nodes appears to be an important prognostic factor and a significant positive correlation between the tumor size and the number of positive axillary lymph nodes is revealed. In the future, it will be necessary to evaluate more potentially useful factors in a prospective fashion using standardized assay and statistical methodology.

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∗ Organizacija kao autor

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∗ Sažetak u časopisu

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Knjige i druge monografije

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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.

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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.

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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.

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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.

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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.

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– 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.

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