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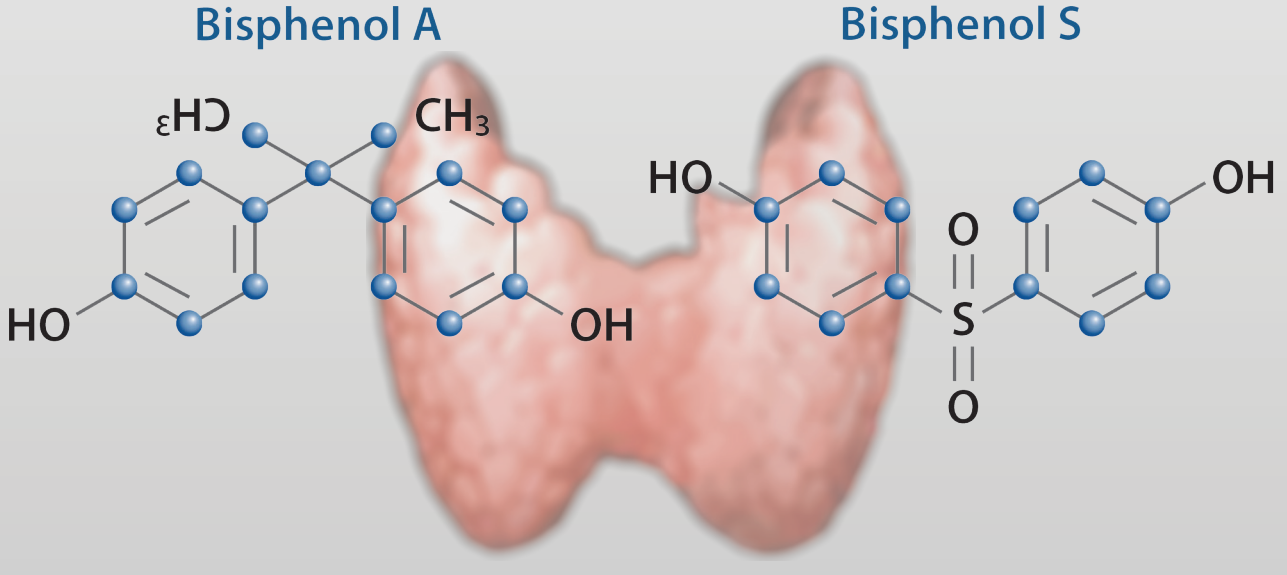

Warning: a major gland is in peril.

(See paper by Leonidas H. Duntas)

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Editorial

WARNING:
A MAJOR GLAND IS IN PERIL

Endocrine disrupting chemical (EDC) is every substance that interferes with hormonal action. The thyroid is more prone to disruption than any other endocrine gland. Different environmental chemicals are negatively influencing the function of thyroid axis at every level. Plastic and its compounds, phthalates, bisphenol A (BPA) and bisphenyl S (BPS) are widely used for food storage. Exposure is the earliest possible through necessary medical devices in hospitalized neonates. Present in catheters in Intensive Care Units, in baby bottles, caned drinks, even in store receipts and recycled paper, they are ubiquitous in environment and almost inevitable. BPS, the substitute for toxic BPA is far from being safe. One of the most significant findings is that low doses can be more harmful than larger ones.

Thyroid hormones have pleiotropic effects, playing critical roles in early brain development, thus influencing intelligence, stimulate growth hormone syntesis, secretion and signalisation, allowing normal somatic growth, bone and pubertal maturation, fertility and the mRNA synthesis of more than hundred proteins with regulatory effect of each and every bodily function. So the question arises; are we less intelligent and less fertile because of abundant use of such chemicals in quotidienne life. Nevertheless, many clinical and experimental studies documented the deleterious effect of chemicals on thyroid gland provoking thyroid dysfunction and rising the incidence of autoimmune thyroid dysorders.

As great admirer of scientific and professional work of Professor Leonidas H Duntas, one of the most prominent contemporary world endocrinologists and thyroidologists, I have the special privilege and honour to present you the Invited review authored by Prof Duntas on this very item with challenging title and inspiring content. Besides his professorship in Athens and Ulm, Prof Duntas is a visiting professor at the Medical Faculty in Belgrade and Novi Sad and Secretary-elect of the European Thyroid Association.

This review aims to sound as alarm and thus call to action all academic organizations to counteract a threat which is disrupting not only the thyroid, but also the life on the earth.

„Feeble though we may seem, we have the power to influence the course of our planet...“

Colin Hiram Tudge

...taken from Duntas L.H. Chemical contamination and the thyroid. Endocrine 2015; 48: 53-64.

Editor-in-Chief

Ljiljana Šaranac

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Invited Review Article

THE THYROID UNDER THREAT IN A WORLD OF PLASTICS

Leonidas H. Duntas

Evgenidion Hospital, Unit of Endocrinology, Metabolism and Diabetes, Thyroid Section, University of Athens, Greece

Abstract. *Among the various categories of thyroid disruptors, plasticizers, particularly phthalates and bisphenol A and substitutes, are most frequently examined due to their very extensive use and extreme durability. Both experimental and clinical studies have shown the deleterious effects of plasticizers on, among other major organ systems, thyroid physiology and thyroid hormone metabolism. Though the mechanism(s) are not as yet well clarified, it is hypothesized that plasticizers exert a suppressive effect on thyroid function and disrupt thyroid signaling. Similar effects have been reported in wildlife, which is also increasingly exposed to the plastic contamination of both solid and aqueous environments. By presenting the results of several recently published large studies linking plastics to thyroid dysfunction, this review aims to sound the alarm and thus call to action all academic organizations in order to counteract a threat which imperils not only the thyroid and the reproductive system but also the entirety of life on our planet.*

Key words: *thyroid, TSH, phthalates, bisphenol-A, endocrine disruptors, hypothyroidism*.

Introduction

Contemporary technological progress has, over the past century, introduced as many as 85,000 new chemicals, without, however, the existence of adequate elimination systems to cope with this onslaught [1]. Thus, with regard to the impact on the human organism, multiple toxins are now entering the body through the air and water, but also via such products as food, makeup and plastic items, incurring an estimated $359 billion a year in healthcare costs. The presentday overwhelming accumulation in our environments of toxic disruptors of endocrine systems (endocrine disruptors or EDs), particularly of the thyroid (and, by extension, of reproductive function), has impelled thyroidologists to identify and categorize a wide array of toxins affecting thyroid function for the purpose of pressing for remedial action [2,3]. Toxic interference of thyroid function can occur at several levels, including iodine uptake, thyroid hormone (TH) production and TH metabolism and/or action, and several recent reviews have analytically enumerated these endocrine disruptors and recorded their highly detrimental effects [4-6].

This brief review aims to underscore the degree to which plasticizers, particularly phthalates and bisphenol A, seriously affect thyroid function in humans, as well as in animals, thereby sounding the alarm as to the grave impact of the thousands of plastics that are today flooding all planetary environments.

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Plasticizers

Phthalates and the thyroid

Plasticizers are synthetic polymers, most commonly polyvinyl chloride (PVC), added especially to rubbers and resins, to improve the flexibility, transparency and durability of the material. Plasticizers used in PVC and other plastics are usually esters of polycarboxylic acids, these including sebacates, adipates, phthalates and terephthalates, azelates, as well as a number of other blends.

Phthalate esters are commonly used in a variety of household applications such as shower curtains, flooring, food containers, wrappers, cleaning materials and personal-care products, including perfumes, eye shadow, nail polish, liquid soap and hair spray [7], also footwear, sporting goods, toys, numerous baby items, car interiors, medical devices. Because they are not chemically bonded to the host plastics, phthalates, esters of phthalic acid, of which the most widely used are di(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DIDP) and diisononyl phthalate (DINP), can easily be released by heating or by extraction and, when absorbed in the body become EDS. The phthalates DIDP and DINP enter the human body via ingestion, inhalation and dermal absorption, while the main source of DEHP is the diet, and particularly fatty foods, milk, butter and meats. Exposure to DEHP also takes place in hospital environments where DEHP leaches directly into liquids that go through PVC/DEHP tubing and equipment. Interestingly, despite the common belief, exposure to phthalates is greater from ingestion of certain foods than through bottled water [8]. Meanwhile, two studies conducted in the USA and Norway between 2003 and 2010 analyzing data from 9,000 individuals found that those who frequently consumed the highly processed,

packaged and handled food from fast food restaurants and junk food hangouts had significantly higher levels of two separate phthalates, DEHP and DINP, in their urine samples [9, 10]. Since these chemicals leach into fast food and junk food from a variety of sources, e.g. packaging, cans, food gloves, this type of eating exposes the public to EDs.

Over the past decade, a number of regulations have been implemented with regard to numerous products containing phthalates, with DEHP having been phased out in Europe by Annex XIV of the European Union's REACH legislation. Under REACH, DEHP can only be used in specific cases if authorization has been granted. Authorizations are granted by the European Commission after obtaining the opinion of the Committee for Risk Assessment (RAC) and the Committee for Socio-economic Analysis (SEAC) of the European Chemicals Agency (ECHA).

Phthalates, albeit inconsistently, have been implicated in thyroid dysfunction and disease. In a very recent population-based cross-sectional study of 279 Taiwanese adults and 79 minors, urine phthalate metabolites were measured together with serum indicators of thyroid function [12]. Serum T4 levels were negatively associated with urinary mono-phthalate and the sum of urinary DEHP metabolite levels. Free thyroxine (FT4) levels were negatively associated with urinary mono-ethylhexyl phthalate (MEHP) levels but positively associated with urinary mono-phthalate, indicating that phthalates may impact thyroid function, even though causality was not definitively identified.

In another study with 6,003 participants from the Korean National Environmental Health Survey (KoNEHS), 2012-2014, DEHP metabolites, along with benzyl-butyl phthalate (BBzP) and di-butyl phthalate (DBP) metabolites and BPA were measured in urine [13]. Association studies were also performed with TH and TSH levels quantified in serum. In general, urinary phthalate metabolites were associated with lowered total T4 or T3 or increased TSH levels in serum. When grouped by sex, urinary metabolites of phthalates were inversely associated with T4 only among males. Among females, mono-benzyl phthalate (MBzP) and mono-n-butyl phthalate (MnBP) levels were inversely associated with TSH and T3, respectively. In addition, a negative association between BPA and TSH was observed.

In China, a recent cross-sectional study was carried out in 216 children aged 5-7 years. Separate collections were made of urinary concentrations of eight mono-phthalate metabolites (MPAEs) from children from both urban and rural areas and an investigation was conducted into their associations with thyroid function and growth hormones [14]. It was ascertained that children from urban areas had higher MPAE concentrations than those from rural areas, while the majority of MPAEs were positively associated with free triiodothyronine (FT3) and FT4. Moreover, insulin-like growth factor 1 (IGF-1) concentration variably diminished by between 0.082ng/mL and 0.132ng/mL with each 1ng/mL increase in phthalate metabolites, while the concentration of insulin-like growth factor binding protein 3 decreased by 0.01mg/L with each 1ng/mL increase in MPAE. The latter results point to the possibility that exposure to certain phthalates in childhood could interfere with TH and growth.

In rodents, the effects of short-term fetal exposure to phthalates on the male reproductive system were unequivocally shown; however, information on the long-term effects of DEHP in utero exposure on gonadal function and other organs are at present sparse [15].

Other animal and wildlife studies have presented novel findings suggesting that in various species DEHP exerts more complex and wider disruptive effects on the endocrine system, including the thyroid and metabolism. For example in fish, numerous environmental stressors exert variable effects, depending on the duration of exposure, on the fish thyroid cascade, these effects possibly mediated via imbalance of plasma T4 and T3 levels or damage to the structure of thyroidal tissues [16]. The thyroidal system in vertebrates is strongly linked to other endocrine systems including the control of reproduction. Therefore, any chemical interference in fish thyroid function due to environmental stressors can potentially have highly damaging effects on several facets of reproduction: this includes inhibition of sperm production as well as decrease of egg production, gonad development, ovarian growth, swimming activity and fertilization and, finally, an increase in larval mortality [16].

Bisphenol A, bisphenol S and the thyroid

Bisphenol A (BPA), a high volume chemical compound of polycarbonate plastic that is widely used for food storage, is likely to be harmful to both human and animal life, most particularly after contact with hot liquids. BPA, as well as phthalates, can leach, migrate or off-gas from products over time and enter the body where it is rapidly metabolized and excreted in urine, with an elimination half-life of less than 24 hr [17].

BPA is ingested in high amounts from canned foods, in contrast to minimal intake from non-canned foods [18]. The monotonic association observed between fast food meat intake and BPA, in contrast to the generally low (1-3 ng/g) levels of BPA in fast food components, although not in hamburgers (10, 9 ng/g), indicate that hamburgers may be a major source of BPA exposure [19].

Despite abundant evidence that BPA is contributing to adverse effects on human health and notwithstanding increasing consumer pressure, to date the FDA, though restricting has not yet banned the use of BPA in baby bottles, a decision thought to be based on industry recommendations [20], while the US Regulatory Commission continues to declare that there is an adequate margin of safety. Indeed, any attempt to remove the substance from the food supply has failed, excepting from baby bottles. This constitutes the epitome of illogicality, since the scientific community strongly believes that BPA is an ED and should therefore, logically, no longer be part of the food chain. Recently, bisphenol S (BPS) was introduced as a substitute for BPA in products claiming to be "BPA free". However, BPS is proving to be no less harmful than BPA

(Figure 1), having been detected in many everyday items, from canned drinks to receipt papers [20]. Studies have pointed to its negative impact on the reproductive system as well as its tendency to multiply fat cells, thereby causing obesity and diabetes. Its effects are observed to be related to timing and length of exposure.

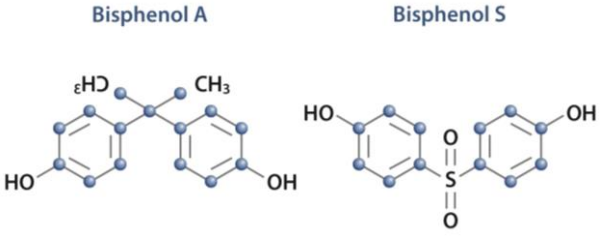

Fig. 1 Chemical structure of Bisphenol A and Bisphenol S

With regard specifically to thyroid signaling, an in vivo and in vitro model (the ZEBRA study) that examined the dose and time effects of BPA on TH synthesis observed altered expression of the genes involved in TH synthesis and of thyroid specific transcriptional factors [21]. In other words, it is likely that BPA exerts direct effects on thyroid follicular cells, albeit the mechanisms, which are complex, are not as yet well clarified [22]. In this line of evidence, it has recently been reported, through the conduct of a series of in vitro and in vivo assays, that BPA, as well as bisphenol F (BPF), another alternative to BPA, interferes with the TH signaling pathway, presumably via a number of different pathways [23]. In the fluorescence competitive binding assay, both BPA and BPF were bound to TH receptors (TRα and TRβ), with an order of magnitude lower than BPA. Furthermore, a BPA substitute, BPS, affects TH homeostasis and TH synthesis at lower doses than does BPA, which could render the substitute more hazardous than BPA [24].

In a cross-sectional study from Thailand, the Thai National Health Examination Survey IV 2009, the relationship between BPA exposure and thyroid function was studied in 2,340 subjects aged 18-94 years [25]. BPA was detected in 52.8 % of serum samples; a significantly negative correlation was found between serum BPA and FT4 levels in males only, while no association was registered with TSH in either gender [25]. In the authors' view, this gender-related association may be linked to androgen-dependent differences in the metabolism of BPA.

In another cross-sectional study from the NHANES 2007–2008 survey, the relationship between urinary phthalate and BPA with serum TH and TSH concentrations was analyzed [26]. While inverse relationships were noted in adults between urinary BPA and total T4 and TSH, significant positive relationships were observed between phthalate metabolites and total T3 among adolescents. Although these study results are not suggestive of any causal relationship a thyroid disrupting effect of BPA can be postulated. This effect may be directly suppressive on thyroid follicular cells and could also be gender- and androgen-dependent.

Animal studies have provided data demonstrating that BPA disrupts TH during pregnancy [27]. Meanwhile, a very up-to-date study in 116 cases of preterm birth and 323 controls showed for the first time in humans that BPA and TSH were inversely associated and that an interquartile range increase in BPA was associated with an 8.21% decrease in TSH: this relation was repeatedly confirmed during the follow-up visits. The issue is a very serious one, since the latter consistent inverse association of BPA with TSH during pregnancy could well affect birth outcome and child development [28].

Another study from China revealed that there is a link between high BPA levels and excessive iodine intake in patients with papillary thyroid carcinoma and nodular goiter (NG) [29]. Though there were no sex-specific differences for total BPA levels measured in serum and in urine and urinary iodine concentrations (UIC), a significant correlation between BPA and UIC was found in those with PTC and NG. The results possibly point to an association between the metabolic pathways of BPA and iodine in the pathogenesis of PTC and NG.

Conclusions

This review of some of the very latest data on plastic contamination worldwide provides unambiguous evidence that the plastic additives, phthalates as well as BPA and its substitutes, are present in a very wide range of consumer products, this resulting in considerable exposure of populations everywhere to these very toxic substances. Studies in humans and in animals have substantiated associations between exposure to phthalates and BPA and serum TH levels, but confirmation on a larger scale is essential. In the meantime, it is incumbent upon the medical community to seriously consider the deleterious effects of BPA (and substitutes) on all body systems and on metabolism and to press for change. On the basis of the steadily increasing evidence of the disruption of thyroid function due to these currently omnipresent toxic chemicals, it is evident that, at the very least concerning all food products, a total ban on BPA and its substitutes must be swiftly implemented.

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Original Article

MONITORING OF IMMUNE RESPONSE IN VIROLOGIC SUCCESSFULLY TREATED HIV-INFECTED PATIENTS IN SOUTHEASTERN SERBIA

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Abstract. *The number of CD4 lymphocytes defines the evolutional stage of HIV-infection and is the most important for a reliable estimation of the individual risk of developing AIDS. However, it is difficult to predict the degree of immune reconstitution during antiretroviral therapy, as it varies significantly from one person to another. Further investigations to better understand the limitations of immunological success are necessary to improve the response to treatment and regimen durability. The current study includes HIV-infected patients in Southeastern Serbia with achieved virologic suppression of HIV infection. The CD4 count was determined by flow cytometry, and was correlated with the duration of treatment, initial number of CD4 cells, type of antiretroviral therapy, mode of transmission of infection, age and gender of examinees. The resulting arithmetic mean and standard deviation of CD4 number was 473±259 cells/µl (range, 1130 cells/µl). There was no statistically significant correlation between the values of CD4 count and length of treatment, stage of the infection at which the therapy was started, treatment profile, method of infection, age or gender. The obtained results are comparable with the existing studies that follow immunological response to antiretroviral therapy and primarily point out the issue of substantial individual response variability, which has not yet been fully elucidated.*

Key words: *HIV, immune response, cART.*

Introduction

During an HIV infection, the complex immunopathogenetic relationship of viral replication, destruction of infected CD4 lymphocytes, and immune response status passes through three phases. The primary infection phase is characterized by a massive viremia, drop of CD4 count, and induction of, primarily, virus-specific cytotoxic lymphocytes. After a period of several weeks up to several months, the parameters find a kind of balance among them and maintain it during the chronic phase of clinical latency. Its highly variable duration, from only a couple of years to even over 15 years, is characterized by progressive anatomical and functional damage to the lymphatic tissue. Eventually, the inability to maintain HIV-specific immune response results in a repeated viral load increase and drop of CD4 count, with the development of the acquired immune deficiency syndrome (AIDS), and presentations of opportunistic infections and tumors.

Combination antiretroviral therapy (cART) substantially reduces the incidence of AIDS and mortality, and now the World Health Organization consolidated guidelines that favor the use of HIV viral load (VL) monitoring for routine identification of its efficacy [1]. However, the monitoring of immunological response to treatment (defined as an increase in CD4 cell count) remains an important clinical tool, being significantly and independently associated with improved prognosis.

Two reference values are generally accepted: above 400-500 CD4 cells/µl, severe AIDS-related diseases are very rare; below 200 CD4 T cells/µl, the risk of AIDS-related morbidity increases with increased duration of immunosuppression.

However, it is difficult to predict the degree of immune reconstitution, as it varies significantly from one person to another. Hence, the precise definition of the success of an immunological treatment does not exist at the moment, and depending on the study it is taken to be an increase of 50, 100 or 150 CD4 T cells/µl per year [2,3].

In such a situation, further investigations to better understand the limitations of immunological success are necessary to improve individual responses to treatment and regimen durability.

Material and Methods

The study included patients on the cART, with achieved virologic suppression of HIV infection (HIV-VL < 50 copies/ml) [4], in whom the number of CD4 cells was determined by flow cytometry (BD FACS Count™).

The patients in whom reduced CD4 lymphocyte counts could be the consequence of concomitant conditions, such as liver cirrhosis, autoimmune diseases, or

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immunosuppressive treatments, were excluded from the study.

Due to possible fluctuations in individual measurements, only those results were taken into account that were consistent with previous CD4 count control measurements, usually performed in six-month intervals.

The obtained results were displayed individually on the scatter diagram, in relation to the duration of treatment, and were also expressed by overall arithmetic mean with standard deviation. The significance of the correlation of their values with treatment duration, initial number of CD4 cells (stage of infection at which cART was started), type of cART, mode of transmission of infection, age and gender of patients, were determined by Student's t-test and a correlation coefficient *r*.

The study was conducted at the Clinic for Infectious Diseases - Clinical Center Niš, the reference center for HIV-infection treatment in the region of Southeastern Serbia.

Results

The study involved 58 patients, 44 (76%) men and 14 (24%) women, with the following age distribution: 12 (20%) aged 20 to 30 years, 21 (36%) aged 30 to 40 years, 13 (23%) aged 40 to 50 years, 8 (14%) aged 50 to 60, and 4 (7%) aged above 60 years of age.

In relation to the mode of transmission, 15 (26%) examinees were infected via intravenous drug abuse, 26 (44%) via homosexual intercourse (MSM), 12 (21%) via heterosexual intercourse, 1 (2%) via blood transfusion, while in 4 (7%) patients the mode of transmission could not be established.

Combination ART was started in 40 (69%) examinees with CD4 lymphocyte counts ≤200/µl, and in 18 (31%) examinees with CD4 counts >200/µl. In 28 (48%) cases, it was based on protease inhibitors (PI), and in 30 (52%) on non-nucleoside reverse transcriptase inhibitors (NNRTI).

Individual results of CD4 count measurements were correlated with the duration of cART, with the final presented value involving all the patients with ≥10 years of treatment (Figure 1). The arithmetic mean with standard deviation of cART duration was 5.72±3.32 years.

The arithmetic mean with standard deviation of CD4 lymphocyte count was 473±259 cells/µl, (range, 1130 cells/µl) (Figure 1). Most of the examinees (33; 57%) had CD4 counts ≤500/µl, while 25 (43%) examinees had CD4 counts ≥500/µl. Of the total number, 10 (17%) examinees had ≤200 CD4 cells/µl, 3 out of 8 patients (37%) at the beginning of treatment (in the first cART year), and 7 (14%) out of the remaining 50 examinees after ≥3 years of treatment.

The association of CD4 cell counts per µl with cART duration was not statistically significant. There was not any statistically significant difference in CD4 counts related to the initial number of CD4 cells ($p > 0,05$), type of cART, mode of transmission of infection, age and gender of patients.

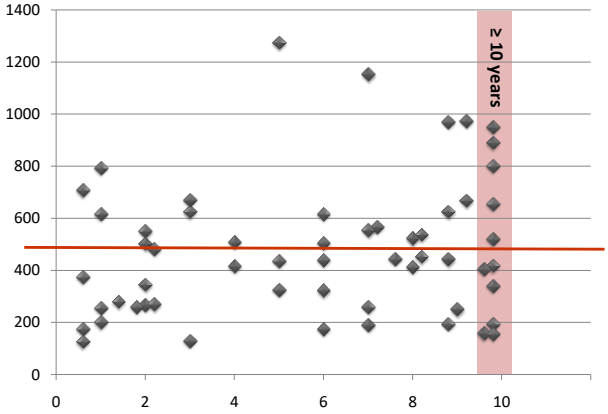

Fig. 1 Individual counts of CD4 cells/µl (y - axis) related to the duration of ART in years (x - axis), with arithmetic mean of CD4 counts in cells/µl.

Discussion

The average number of 467 CD4 cells/µl obtained in the study and absence of complete recovery of the immune system in most of the patients (43% examinees ≥500 cells/µl, 57% examinees <500 cells/µl) corresponds to the results presented in numerous studies of immune reconstitution after multiannual antiretroviral therapy followed by a complete virologic response [5,6,7,8].

Since CD4 counts after several years of treatment achieve a plateau, characterized by small or absent increases, the connection between these two parameters was not evaluated in the above studies. , The present study, on the other hand, included patients at the beginning of cART, when the dynamics of change in CD4 lymphocyte counts was most conspicuous – usually characterized by a rapid increase in the number of circulating CD4 cell lymphocytes during the first 2-3 months of treatment (median of 21.2 cells/µl per month), representing mainly a redistribution of activated CD4 memory cells previously sequestered in the lymphoid tissue and generalized reduction in apoptotic cell death, followed by a slower second phase of CD4 cell expansion (5.5 cells/µl per month), representing the expansion of naive CD4 cells [9].

However, that did not produce any significant change in the overall study results, nor did it reveal any significant association of CD4 lymphocytes with treatment duration. A possible explanation could be, according to recent recommendations, an earlier initiation of treatment (regardless of CD4 count), and now a reduced number of presentations with advanced HIV disease (3 of the 8 patients, i.e. 37%). A smaller percentage of late presenters has also been reported in different European and US studies [10,11,12].

The remaining 7 out of 50 examinees in whom CD4 counts were below 200/µl after multiannual treatments, belonged to the group of immunological non-responders. Their prevalence of 14% was comparable with the results of other studies of such a discordant immune response, where satisfactory immune status could not be

achieved despite prolonged viral suppression [13]. The consequences of this condition in the long run are not yet clear [14,15]. The data from the relevant clinical surveys suggest that mortality seems to be slightly higher, but has not been related to AIDS-defining diseases, the increased incidence of which is present only in the first few months of therapy [16]. The exact background of this discordant response is also unclear, and DHHS guidelines do not recommend switching an otherwise suppressive cART regimen in this patient group [17].

In general, the risk factors for the lack of immunologic response are heterogenous and often unmodifiable. Firstly, a complete reconstitution of the immune system is only rarely possible if the patient's initial situation is poor (the worse the immune system, the more unlikely a complete recovery), and viral suppression over several years cannot change that [6,8,18]. Forty-four percent of patients with less than 100 CD4 cells/µl at the initiation of cART failed to reach 500 CD4 cells/µl even after a decade of virologic successful treatment, while 25% of patients with 100-200 CD4 cells/µl still showed that risk [14,19]. The trials of several thousand patients confirmed a connection of the initial CD4 count with the level of an immune response, as suggested by their correlation as well at the borderline of statistical significance ($p > 0.05$), which was verified by the presented study of a much smaller number of subjects.

Age may also play a role. In older patients, immunological response is often only moderate, mainly due to thymic degeneration [20,21]. This observation has not been confirmed by the present study either, probably due to the same reasons as for the impact of low number of initial CD4 cells. On the other hand, these results in a small number of patients indicate a substantial individual variability of the above-mentioned risk factors for poor immune response.

Other reasons that could possibly explain the wide range of immune responses (1130 cells/µl in the present study), such as gender, mode of transmission of infection, or the difference between NNRTIs- and PI-based cART, were not associated with CD4 counts in the current study, as confirmed in other studies in the past [22,23].

Conclusion

Alongside viral load, measurement of the CD4 cells level is the most important parameter or surrogate marker in HIV medicine. As the strongest predictor of HIV progression, allowing for a reliable estimation of the individual risk of developing AIDS, it is of vital importance for the patients.

Having in mind that variability of CD4 expression among patients is still completely unresolved, further efforts to improve the understanding of the success and failure of an immune response would have implications not only for HIV infection, but could also possibly help in the interpretation of the role of cellular immunity in other diseases.

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Original Article

ONE-YEAR CARDIOVASCULAR OUTCOME IN PATIENTS ON CLOPIDOGREL ANTI-PLATELET THERAPY AFTER ACUTE MYOCARDIAL INFARCTION

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Abstract. *The aim of this study was to determine the risk factors in patients on clopidogrel anti-platelet therapy after acute myocardial infarction, for cardiovascular mortality, re-hospitalization and admission to emergency care unit. We followed 175 patients on dual antiplatelet therapy, with clopidogrel and acetylsalicylic acid, for 1 year after acute myocardial infarction, both STEMI and NSTEMI. Beside demographic and clinical characteristics, genetic ABCB1, CYP2C19 and CYP2C9 profile was analyzed using Cox-regression analysis. End-points used were: mortality, re-hospitalization and emergency care visits, all related to cardiovascular system. During the accrual and follow-up period, 8 patients (4.6%) died, mostly as a direct consequence of an acute myocardial infarction. Re-hospitalization was needed in 27 patients (15.4%), in nine patients (33.3%) with the diagnosis of re-infarction. Thirty-two patients (18.3%) were admitted to emergency care unit due to cardiovascular causes, up to 15 times during the follow-up. NSTEMI was an independent predictor of all three events registered (mortality OR=7.4, p<0.05; re-hospitalization OR=2.8, p<0.05); emergency care visit OR=2.4, p<0.05). Other significant predictors were related to kidney function (urea and creatinine level, creatinine clearance), co-morbidities such as arterial hypertension and decreased left ventricular ejection fraction, as well as clopidogrel dosing regimen. As a conclusion, it may be suggested that one of the most significant predictors of cardiovascular events (mortality, re-hospitalization and emergency care visits) is NSTEMI. Besides, clopidogrel administration according to up-to-date guidelines, with high loading doses and initial doubled maintenance doses, improves 1-year prognosis in patients with AMI.*

Key words: *clopidogrel, acute myocardial infarction, cardiovascular outcome*.

Introduction

Despite improvements in pharmacotherapy and management of patients with acute myocardial infarction, the mortality rate and the incidence of cardiovascular outcomes remain high. In Serbia, mortality is over 10%, partially due to low rate of primary PCI (22%), the most beneficial management option in these patients [1]. The rate of various cardiovascular events is the highest in the first month after the acute event, but remains significant even a year after [2]. Cardiovascular diseases are the most common cause of death in Serbia (53.3%), out of which 18.5% account for ischemic heart disease. More than a half of this mortality (54.0%) is caused my ACS. The highest number of newly diagnosed AMI is among patients older than 75 (34.1%), in both genders. Male and female gender ratio is 1:1.6. The overall incidence rate is 232.2 (or 107.7 when standardized to the world population). The Nis region is characterized with very low standardized incidence rate (below 105.0), but with moderate mortality rate (31.3), the same as the country's average [1]. The majority of the patients with AMI are treated according to the evidence-based guidelines. Nevertheless, pharmacogenetic factors may alter the medication efficacy and worsen the outcome in these patients. Besides, the risk of various cardiovascular events is associated with age and other patients' characteristics, medical history and revascularization

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method [2]. The rate of re-hospitalizations in a 30-day period goes up to 20% [3,4] in about a quarter due to cardiovascular causes [3]. After 3 months, the rate rises to 9.3%, and up to 20.2% after a year. Revascularization is needed in approximately 5% [5]. Known predictors are female sex, CABG surgery or PCI, prior ICD, vascular disease, chronic kidney disease, diabetes mellitus, rheumatic valvular disease, chronic pulmonary disease and anemia [5–7].

Beside patients' characteristics and co-morbidities, primarily decreased, but increased clopidogrel efficacy, as well, may be the reason of poor clinical outcome. P-glycoprotein is a transporter involved in the absorption process of various drugs prescribed in patients with AMI. The presence of polymorphic T allele of *ABCB1* gene may alter their bioavailability, and their pharmacodynamics effects. The absorption of clopidogrel, used in combination with acetylsalicylic acid as dual anti-aggregation therapy, is impaired in *ABCB1 TT* homozygotes, which may account for more than 25% in Caucasians [8]. Besides, interactions with proton-pump-inhibitors prescribed for gastroprotection, carvedilol (and some other ACE-inhibitors), statins and calcium-channel blockers, via P-glycoprotein inhibition are possible [9]. Other polymorphisms of importance are on the genes encoding cytochrome P450 enzymes, particularly CYP2C19 and CYP2C9. Both of them are involved in the clopidogrel transformation into active metabolite. The induction or inhibition of these enzymes, as well as the other CYP involved in clopidogrel biotransformation, such as CYP3A4, may lay in the basis of significant drug interactions [10].

The aim of this study was to determine the risk factors in patients on clopidogrel anti-platelet therapy after acute myocardial infarction, for cardiovascular mortality, re-hospitalization and admission to emergency care unit.

Subjects and Methods

The study was approved by Ethics Committee of the University of Niš, Faculty of Medicine. All the subjects were treated in accordance with the Declaration of Helsinki. Before being recruited, each patient gave an informed consent. It was designed as a prospective cohort study.

Patients included in the study were treated at the Clinic of Cardiology, Clinical Center Niš, Serbia. They were recruited upon acute coronary syndrome with/without ST-elevation diagnosis with biochemical and electrocardiographical confirmation. We followed 175 patients, both with STEMI (75.2%) and NSTEMI (20.9%). Half of the patients (49.7%) were treated with primary PCI. Thirty-six patients (20.6%) were revascularized with fibrinolytics administration, alteplase (18.9%) or streptokinase (1.7%). Among alteplase patients, 7(4.0%) underwent rescue PCI. Conventional therapy was applied to the rest 29.7% patients.

Study group characteristics are shown in Table 1. There were 122 male (69.7%) and 53 female (30.3%) patients. Their age varied from 30 to 89 years (average 60.80±11.33 years).

Table 1 Baseline characteristics of the study patients group

	mean ± SD	
Age (years)	60.81 ±	11.29
Heart rate (1/min.)	79.60 ±	19.08
Systolic blood pressure (mmHg)	130.92 ±	26.81
Diastolic blood pressure (mmHg)	79.79 ±	16.02
Ejection fraction (%)	50.77 ±	11.70
End-systolic dimension (mm)	52.33 ±	6.55
End-diastolic dimension (mm)	37.35 ±	8.07
Right ventricular systolic pressure (mmHg)	34.82 ±	11.71
Left atrial size (mm)	39.76 ±	5.59
Body-mass index (kg/m^2)	26.99 ±	3.91
Cholesterol (mmol/l)	5.58 ±	1.37
LDL (mmol/l)	3.62 ±	1.19
HDL (mmol/l)	1.12 ±	0.58
Triglycerides (mmol/l)	2.01 ±	2.68
Hemoglobin (g/l)	138.91 ±	19.51
Hematocrit (l/l)	0.41 ±	0.06
Platelets (10^9/l)	253.03 ±	78.75
Leukocytes (10^9/l)	11.82 ±	10.91
Erythrocytes (10^{12}/l)	4.69 ±	0.65
AST (U/l)	138.52 ±	170.49
ALT (U/l)	45.25 ±	49.47
LDH (U/l)	934.33 ±	769.07
Glucose(mmol/l)	8.28 ±	4.56
Creatinine (μmol/l)	103.41 ±	44.72
Creatinine clearance (ml/min.)	79.28 ±	25.71
Urea (mmol/l)	6.76 ±	3.20
Sodium (mmol/l)	136.33 ±	10.31
Potassium (mmol/l)	4.33 ±	0.48
CRP (mg/l)	18.28 ±	32.55
Troponins (ng/l)	12.82 ±	28.47
Creatine kinase (U/l)	1052.74 ±	1403.40
CKMB (U/l)	100.86 ±	126.35
Fibrinogen (g/l)	4.63 ±	2.06
	N	(%)
Male	122	(69.7%)
STEMI	135	(77.1%)
Previous AMI	31	(17.7%)
Hypertension	110	(62.9%)
DM	49	(28.0%)
Atrial fibrillation	16	(9.1%)
Smoking	60	(34.3%)

Pharmacological therapy was carried out according to the guidelines. All the patients received antiplatelet and anticoagulant drugs. Antiaggregation therapy was achieved with ASA (98.3%) and clopidogrel (100.0%). The therapy of AMI consisted of ACE inhibitors (68.4%), beta-blockers (75.4%), diuretics (20.6%), spironolactone (17.7%), calcium channel blocker (11.4%), amiodarone (10.3%), digoxin (6.3%), long-lasting vasodilators (24.6%) and trimetazidine (10.3%), as well. Besides, they were given lipid-lowering statins (94.3%), pantoprazole for gastroprotection (90.3%) and anxiolytics (33.7%).

Table 2 Primers used for SNPs detection

ABCB1 C3435T	C (5'-GGTGTCACAGGAAGAGATC-3') T (5'-CAGCCGGGTATAGTCACAGGAAGATATT-3') Reverse (5'-GGCCAGAGAGGCTGCCACAT-3')
CYP2C19*2	Forward (5'-AATTACAACCAGAGCTTGGC-3') Reverse (5'-TATCACTTTCCATAAAAGCAAG-3')
CYP2C19*17	Forward (5'-GCCCTTAGCACCAAATTCTC-3') Reverse (5'-ATTTAACCCCCTAAAAAAACACG-3')
CYP2C9*2	Forward (5'-GTATTTTGGCCTGAAACCCATA-3') Reverse (5'-GGCCTTGGTTTTTCTCAACTC-3')

Thirty-one patients (17.7%) had previous AMI (re-infarction patients were excluded from the study). Among co-morbidities, 110 (62.9%) had HTA diagnosed for 11.29±8.61 years, while 49 (28.0%) suffered from diabetes mellitus type 2 for approximately 8.64±7.83 years, and 16 patients (9.1%) had atrial fibrillation. Anamnestic data showed that one third of the patients (34.3%) were current smokers (former smokers were considered non-smokers).

Accrual time was 1 year, while the follow-up time was 6 months. During that period patients were monitored for reporting to the emergency unit, re-hospitalizations and mortality. Non-related non-cardiovascular events were not included into analysis.

During the accrual and follow-up period, all the patients were genotyped for ABCB1 C34335T (rs1045642), CYP2C19*2 (rs4244285) and *17 (rs12248560), as well as CYP2C9*2 (rs1799853). From each collected whole blood sample, we have isolated genomic DNA manually. Using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method and specific primers (Table 2), before-mentioned small nuclear polymorphisms (SNPs) were detected.

Statistical Package for Social Sciences (SPSS 16.0; Chicago, Ill., USA) was used for statistical data analysis. Baseline characteristics are presented as frequencies or means with SDs. Quantitative variables were compared between groups using Student's t-test, while for qualitative variables Fisher's exact test was performed. A p value <0.05 was considered to be a measure of statistical significance. Using Cox-regression, hazard ratios and 95% CIs were calculated, and therefore, potentiated the comparison of outcome occurrence between groups and the effect of each predictor.

Results

During the accrual and follow-up period, 8 patients (4.6%) died. In 6 cases it was the direct consequence of an acute myocardial infarction. The other two deaths both had cardiovascular cause: infective myocarditis and lower extremity thrombosis. They occurred between 7 and 297 days after the recruitment. Re-hospitalization was needed in 27 patients (15.4%). Most of them were re-hospitalized only once during the follow-up period, while the maximum number of re-hospitalizations was 7 in one case. Nine patients (33.3%) were re-hospitalized with the diagnosis of re-infarction. In one of the cases the re-infarction was caused by stent thrombosis. The symptoms of angina pectoris, stable (22.2%) or unstable (11.1%), were the reason for re-admission reported as well. Thirty-two patients (18.3%) were admitted to emergency care unit due to cardiovascular causes, up to 15 times during the follow-up.

ABCB1 C3435T genotyping was 100.0% successful, while in 51 patients (29.1%) we could not determine CYP2C19 and CYP2C9 polymorphism. The observed genotype frequencies did not deviate significantly from those expected at Hardy-Weinberg equilibrium (Table 3).

Table 3 ABCB1 C3435T, CYP2C19 and CYP2C9 genotype frequencies

Gene	Genotype	N (f)	χ^2 (p)
ABCB1 C3435T	CC	41 (23.4%)	
	CT	81 (46.3%)	0.658 (0.720)
	TT	53 (30.3%)	
CYP2C19	PM	5 (4.0%)	
	IM	33 (26.6%)	4.649 (0.460)
	EM	46 (37.1%)	
	URM	40 (32.3%)	
CYP2C9	CC	115 (79.3%)	
	CT	28 (19.3%)	4.634 (0.099)
	TT	2 (1.4%)	

Table 4 Cardiovascular outcomes in relation to genotype

	Emergency care unit admission (HR (CI 95%), p)	Re-hospitalization (HR (CI 95%), p)	Cardiovascular death (HR (CI 95%), p)
ABCB1 genotype (CC-CT-TT)	1.2 (0.7-1.9), 0.526	1.4 (0.9-2.5), 0.170	0.5 (0.2-1.4), 0.221
ABCB1 TT genotype	0.9 (0.4-2.0), 0.866	1.3 (0.6-2.8), 0.556	0.3 (0.0-2.7), 0.298
ABCB1 any T allele	1.8 (0.7-4.8), 0.216	2.7 (0.8-9.1), 0.102	0.5 (0.1-2.1), 0.348
CYP2C19 phenotype (SM-IM-EM-URM)	0.8 (0.5-1.2), 0.245	0.9 (0.5-1.4), 0.528	0.8 (0.4-2.0), 0.694
CYP2C19 any *2 allele	1.5 (0.7-3.3), 0.309	1.4 (0.6-3.4), 0.442	1.8 (0.4-7.9), 0.460
CYP2C19 any *17 allele	0.8 (0.4-1.8), 0.612	1.0 (0.4-2.5), 0.885	0.5 (0.1-2.7). 0.448
CYP2C9 any *2 allele	0.4 (0.1-1.3), 0.128	0.7 (0.2-2.1), 0.553	0.6 (0.1-5.4), 0.686

As genetic predictors, we have analyzed the presence of various genotypes and phenotypes of ABCB1, CYP2C19 and CYP2C9 genes. For each of the three outcomes monitored, none of the predictors were shown to be statistically significant (Table 4).

We have tested the patients' characteristics, co-morbidities and social habits such as smoking as risk predictors of cardiovascular outcomes (Table 5–7). Even though univariate Cox-regression analysis have identified a number of statistically significant predictors of need for re-hospitalization (Table 5), after their inclusion into a multivariate model (χ^2=18.522, p<0.001), the only predictors that remained independently significant were the type of AMI, with or without ST-segment elevation, and clopidogrel dosing regimen. Patients after NSTEMI had 2.8 higher risk (p<0.05) of re-hospitalization due to cardiovascular indications. Patients with lower dose clopidogrel dosing regimens (lower or no loading dose) had 2.3 times higher risk of re-hospitalization (p<0.05).

Considering emergency care unit admission, due to cardiovascular symptoms and signs (Table 6), the majority of predictors identified by univariate Cox-regression modeling, remained independently significant in the multivariate model (χ^2=35.662, p<0.001). In comparison to STEMI, NSTEMI is associated with 2.4 times higher risk (p<0.05). High LDL (above 3mmol/l) leads to 2.5 times lower risk (p<0.05). Patients with decreased LVEF after AMI, below 45%, had 3 times (p<0.05) higher risk of need for emergence care. Increased creatinine concentration, for each 10μmol/l, increases the risk 1.1 times (p<0.001). Patients with HTA are at 3 times higher risk (p<0.05) of emergency care need. Among the drugs prescribed, therapy with calcium channel blockers increased the risk by 2.8 times (p<0.05).

Despite low mortality, we have identified (Table 7) two significant predictors in a multivariate Cox-regression model (χ^2=15.137, p<0.01). Acute myocardial infarction without ST-segment elevation was associated with 7.4 higher risk of cardiovascular mortality (p<0.05). The increase in urea concentration for each unit augments the risk 1.1 times (p<0.05).

Table 5 Predictors of re-hospitalization due to cardiovascular causes

Re-hospitalization (HR (CI 95%), p)	Univariate Cox-regression	Multivariate Cox-regression
Age (years)	1.041 (1.005-1.078), 0.024	
NSTEMI vs. STEMI	4.148 (1.948-8.829), 0.000	2.785 (1.102-7.038), 0.030
High LDL>3mmol/l	0.407 (0.176-0.942), 0.036	
Hemoglobin (g/l)	0.982 (0.965-1.000), 0.047	
Leukocytes (10^9/l)	0.817 (0.679-0.983), 0.032	
AST (U/L)	0.994 (0.988-0.999), 0.032	
Creatinine (μmol/l)	1.006 (1.000-1.011), 0.035	
Urea (mmol/l)	1.095 (1.019-1.178), 0.014	
CK(U/L)	0.999 (0.999-1.000), 0.049	
Previous AMI	4.102(1.919-8.771), 0.000	
HTA	5.069 (1.526-16.835), 0.008	
DM type 2	2.214 (1.036-4.732), 0.040	
Clopidogrel loading dose (mg)	0.996 (0.994-0.998), 0.000	
Clopidogrel loading dose/BMI (mg·m^2/kg)	0.905 (0.838-0.977), 0.011	
Clopidogrel dosing regimen (mg)	2.356 (1.227-4.522), 0.010	2.284 (1.136-4.593), 0.020
Carvedilol vs. Bisoprolol	3.060 (1.099-8.518), 0.032	
Diuretic	2.927 (1.358-6.308), 0.006	
Spironolactone	2.835 (1.298-6.192), 0.009	
Long lasting nitrates	2.557 (1.196-5.463), 0.015	

Table 6 Predictors of emergency care due to cardiovascular causes

Emergency care unit admission (HR (CI 95%), p)	Univariate Cox-regression	Multivariate Cox-regression
NSTEMI	3.071 (1.512-6.240), 0.007	2.379 (1.080-5.243), 0.031
High LDL>3mmol/l	0.431 (0.205-0.907) 0.027	0.399 (0.181-0.881), 0.023
EF<45%	2.395 (1.191-4.817), 0.014	3.044 (1.398-6.629), 0.005
Creatinine (μmol/l)	1.009 (1.004-1.014), 0.001	1.011 (1.004-1.017), 0.001
Urea (mmol/l)	1.152 (1.067-1.243), 0.000	
HTA	2.867 (1.179-6.969), 0.024	3.133 (1.200-8.180), 0.020
Diuretic	2.479 (1.212-5.072), 0.013	
Calcium channel blocker	2.664 (1.151-6.168), 0.022	2.782 (1.000-7.738), 0.050

Table 7 Predictors of cardiovascular mortality

Cardiovascular mortality (HR (CI 95%), p)	Univariate Cox-regression	Multivariate Cox-regression
NSTEMI	11.019 (2.223-54.615), 0.003	7.431 (1.371-40.267), 0.020
LDL (mmol/l)	0.514 (0.281-0.940), 0.031	
High LDL>3mmol/l	0.217 (0.049-0.970), 0.046	
Hemoglobin (g/l)	0.966 (0.938-0.995), 0.021	
Creatinine (μmol/l)	1.009 (1.003-1.015), 0.004	
Creatinine clearance (ml/min.)	0.933 (0.886-0.983), 0.009	
Urea (mmol/l)	1.162 (1.073-1.257), 0.000	1.094 (1.001-1.195), 0.046
Previous AMI	4.820 (1.205-19.277), 0.026	
Clopidogrel loading dose (mg)	0.994 (0.990-0.999), 0.016	
Clopidogrel dosing regimen (mg)	5.190 (1.166-23.107), 0.031	
Acetylsalicylic acid loading dose (mg)	0.993 (0.986-0.999), 0.034	
Calcium channel blocker	5.004 (1.195-20.946), 0.027	

Discussion

After a 1-year follow-up of patients after AMI, 4.6% died, 15.4% were re-hospitalized and 33.3% visited emergency unit, all associated with cardiovascular events. These rates are slightly lower than those reported in previous studies (1,3–5). One of the risk factors for poor clinical outcome, regarding all three events registered is NSTEMI. Compared to STEMI patients, NSTEMI patients are associated with 2.4 times higher risk of emergency care needed, 2.8 times higher risk of re-hospitalization, and even 7.4 times higher risk of cardiovascular death. It is suggested that STEMI patients are favored by rapid revascularization and more aggressive pharmacotherapy, resulting in lower rate of mortality and re-hospitalizations in 1-year period after AMI [11].

Elevated creatinine concentration was found to be and independent predictor of re-hospitalizations, while elevated urea concentration was associated with increased risk of cardiovascular mortality. Patients with chronic kidney disease, especially those with more severe stages, are less likely to be revascularized and prescribed evidence-based therapy. On the other hand, they presented more often with bleeding complications. When summed up, this results in higher mortality rate, particularly in STEMI patients [12].

Higher clopidogrel dosing regimen was found to be associated with lower risk of re-hospitalizations. When higher clopidogrel doses are administered, the production of clopidogrel active metabolite is multiplied, despite some saturation of the pharmacokinetic processes involved [13]. In the elderly, no loading dose is recommended, while lower loading dose is prescribed in patients administered fibrinolytics [14]. High loading dose gives the greatest benefit in the first days after AMI, but increased maintenance dose, recommended in the first week, significantly decreases the risk of major adverse cardiovascular events [15].

Hyperlipidemia is a known risk factor for ACS and for the worse prognosis after AMI [16]. Our results have shown that high LDL favored good prognosis, as found in similar studies [17]. This may be due to the therapy with statins. Beside lowering LDL, these drugs have a number of pleiotropic effects in atherosclerotic diseases. Statins may have antithrombotic effect, directly diminishing the possibility of various cardiovascular events, due to inhibited platelet and activation and procoagulant protein tissue factor expression [18]. Besides, there are significant changes in atherosclerotic plaque characteristics in patients receiving statin therapy, concerning calcium and fibrous deposition, as well as its elastic membrane, thus stabilizing the plaque [19].

After suffering AMI, in a large number of patients there is a decrease in left ventricular EF. This worsening of the cardiac contractile function is one of the prognostic factors for bad prognosis. The risk increases at least 3 times [17,20], as found in our results. Mild and severe left ventricular dysfunctions are associated with higher risk in patients with AMI, especially in combination with co-morbidities [21].

We have found patients with HTA to be at more than 3 times higher risk of suffering from cardiovascular symptoms during the follow-up period after AMI. In the previous studies, somewhat lower hazard risk ratio was found for these patients to experience major adverse cardiovascular events [22,23], but it may rise to 5 times higher risk in patients with unspecified chest pain [24]. Therefore, patients on antihypertensive drugs, such as calcium channel blockers, were associated with worse prognosis, as well.

Although there are numerous studies suggesting the association between genetic profile, including cytochrome P450 enzymes and P-glycoprotein, and outcome in patients after AMI [25–27], we have not obtained statistically significant results. This may be explained by insufficient number of events for such genetic analysis. Except for ABCB1 polymorphisms for P-glycoproteins, low-function genotypes are rare in the Caucasian population.

Conclusion

As a conclusion, we may imply that one of the most significant predictor of cardiovascular events (mortality, re-hospitalization and emergency care visits) is NSTEMI. Besides, clopidogrel administration according to up-to-date guidelines, with high loading doses and initial doubled maintenance doses, improves 1-year prognosis in patients with AMI.

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Original Article

ASSESSMENT OF CARDIOVASCULAR RISK AND COMORBIDITY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Abstract. *High rates of morbidity and mortality in patients with chronic diseases of the kidney are for the most part caused by the high prevalence of cardiovascular diseases and high rates of fatal cardiovascular events. The aim of the study was to establish the prevalence and distribution of cardiovascular risk factors in patients with chronic kidney diseases, in various stages of chronic renal failure. The examinees were classified into three groups based on the level of glomerular filtration rate: over 60 ml/min/1.73m^2; 30-59 ml/min/1.73m^2; and 15-29 ml/ min/1.73m^2. Traditional risk factors of age, hypertension, systolic blood pressure, glycemia, diabetes, serum level of total cholesterol and triglycerides, triglyceridemia, and hypertrophy of the left ventricle showed a significantly positive rising trend of their mean values or prevalence, inversely dependent upon the level of declining glomerular filtration rates. Mean values of serum HDL cholesterol level demonstrated a significant declining trend, concomitant with decreasing glomerular filtration rate. The prevalence of hypercholesterolemy, smoking and obesity, as well as the mean value of body mass index, showed significant intergroup variations, but without any continuing trend related to glomerular filtration rate. Non-traditional risk factors of anemia, proteinuria, and hypoalbuminemia showed a significant rising trend of prevalence inversely dependent upon the degree of reduction of glomerular filtration rate. The levels of hematocrit and serum albumins showed a positive correlation with the reduction of glomerular filtration rate. In pre-dialysis patients with chronic kidney diseases, a high prevalence of the studied cardiovascular risk factors was found. Cardiovascular risk progressively rises with decreasing glomerular filtration rate, being significantly elevated as early as the initial stages of renal failure.*

Key words: *chronic kidney disease, cardiovascular risk, cardiovascular morbidity*.

Introduction

Chronic kidney disease (CKD) patients are patients with high morbidity and mortality rates. The high mortality rate is for the most part caused by high prevalence of cardiovascular diseases and high rates of fatal cardiovascular events in CKD patients. Individuals with CKDs are at a higher risk of dying of cardiovascular diseases thanof developing terminal kidney failure [1,2].

CKD patients have a three times higher incidence of classical, i.e. traditional cardiovascular risk factors compared to the general population. Moreover, "non-traditional" cardiovascular risk factors and specific changes in CKDs are considerably involved in the pathogenesis of cardiovascular diseases in chronic uremia: hypervolemia and renin-angiotensin-aldosteron system activation, hyperhomocysteinemia, oxidative stress, chronic inflammation and malnutrition (MIA syndrome), secondary hyperparathyroidism and abnormal metabolism of calcium and phosphorus, disturbed acid-base balance, anemia, thrombotic system alteration, direct effects of uremic toxins [3,4].

Aim of the Study

The aim of the study was to establish the prevalence and distribution of cardiovascular risk factors in CKD patients in different stages of chronic renal failure.

Method

The study involved 205 CKD patients in the pre-dialysis stage, aged 55.1±4.9 years, at the Department of Nephrology with Dialysis Center, General Hospital Leskovac (Table 1).

The examinees were divided into three groups based on the level of their renal function, i.e. their glomerular filtration rate (GFR):

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Table 1 basic patient characteristics

	Total number of examinees 205	>60 ml/min 60 / 29.3%	30-59 ml/min 86 / 42.0%	15-29 ml/min 59 / 28.8%
Age (in years)	55.1 ± 4,9	52.3 ± 7,7	59.7 ± 8,9	63.0 ±4.5
<45	19.5% (40/205)	23.3% (14/60)	19.8% (17/86)	15.3% (9/59)
45-65	42.0% (86/205)	33.3% (20/60)	43.0% (37/86)	40.2% (29/59)
>65	38.5% (79/205)	43.3% (26/60)	37.2% (32/86)	35.6% (21/59)
Gender				
men	48.8% (100/205)	51.7% (31/60)	47.7% (41/86)	47.5% (28/59)
women	51.2% (105/205)	48.3% (29/60)	52.3% (45/86)	52.5% (31/59)
BMI (kg/m^2)	28.0 ± 4.5	26.8 ± 3.8	29.4 ± 4.2	28.6 ± 4.4
Kidney disease				
glomerulo(pyelo)nephritis	15.6% (32/205)	8.3% (5/60)	19.8% (17/86)	16.9% (10/59)
nephro(angio)sclerosis	11.2% (23/205)	18.3% (11/60)	8.1% (7/86)	8.5% (5/59/)
diabetic nephropathy	19.0% (39/205)	23.3% (14/60)	18.6% (16/86)	15.3% (9/59)
policystic kidney disease	6.8% (14/205)	11.7% (7/60)	4.7% (4/86)	5.1% (3/59)
endemic nephropathy	4.4% (9/205)	8.3% (5/60)	2.3% (2/86)	3.4% (2/59)
urologic disease	12.7% (26/205)	20.0% (12/60)	12.8% (11/86)	5.1% (3/59)
other/unknown	30.2% (62/205)	16.7% (10/60)	33.7% (29/86)	39.0% (23/59)

BMI – body mass index

1. slightly reduced GFR (over 60 ml/min/1.73m^2) - 60 examinees – group A;
2. moderately reduced GFR (30-59 ml/min/1.73m^2) - 86 examinees – group B;
3. significantly reduced GFR (15-29 ml/min/1.73m^2) - 59 examinees – group C.

The prevalence of complications and comorbid conditions in chronic renal failure start to grow with GFR reduced below 60 ml/min/1.73m^2, so that most researchers, studies, and clinical guidelines tend to consider this value as the point of onset of hronic renal failure. The group of examinees with GFR of 60 ml/min/1.73m^2 and higher was taken as the reference group in further analyses and comparisons [5–8].

Due to the fact that the MDRD equation and Cockroft-Gault formula have got numerous limitations, especially when cardiovascular patients are concerned, GFR was determined in this study via the mean values of creatinin clearance in at least two samples of 24h urine creatinine excreation and serum creatinine. The same 24h urine was also used for proteinuria measurements.

The following variables were considered the traditional cardiovascular risk factors: male gender, elevated blood pressure, obesity, diabetes, hypercholesterolemia, low HDL cholesterol, hypertriglyceridemia, left ventricular hypertrophy (LVH), smoking, as well as age, systolic and diastolic blood pressure values and body mass index (BMI). Hypertension was defined as the values of systolic pressure ≥140 mmHg and diastolic pressure ≥90 mmHg or the use of antihypertensive agents. Hypercholesterolemia was defined as the serum fasting cholesterol ≥6.5 mmol/l, ≥5.0 mmol/l in the cases of an earlier myocardial infarction or the patient using antilipemic drugs. Left ventricular hypertrophy was defined, based on the echocardiographic test, as a thickness of the posterior left ventricle wall of over 11 mm, and/or interventricular septal thickness of over 12 mm. The criterion for diabetes was fasting serum glucose ≥7.0 mmol/l, serum glucose without fasting, postprandial, ≥11.1 mmol/l, or the use of oral antidiabetic agents. Obesity was defined as the body mass index (BMI) >30 kg/m^2. BMI was calculated as the ratio of body weight (in kg) and body surface (in m^2) calculated from nomogram tables via body height. Smoking status was defined as active smoker or the period of smoking of less than a year before the onset of the study.

Among the non-traditional cardiovascular risk factors we included proteinuria, anemia, and hypoalbuminemia, as well as the mean values of hematocrit, leukocytes in the blood, serum albumin and fibrinogen.

Cox logistic regression was used to examine the association of cardiovascular risk factors with glomerular filtration rates. Chi-square and Wilcoxon tests were used to establish the prevalence and distribution of variables.

Results

The values were presented as absolute in the form of mean values with standard deviation or in percentage form, i.e. the prevalence of cardiovascular risk factors. The data were presented for the total number of patients and by groups A, B, and C, based on the level of renal function.

Table 2 presents the data for traditional cardiovascular risk factors.

The following variables showed a significant positive rising trend of mean values or prevalence, inversely dependent upon the degree of decrease of GFR values: age, hypertension, systolic blood pressure, glycemia, diabetes, serum levels of total cholesterol and triglycerides, triglyceridemia, and LVH. Serum HDL cholesterol level showed a significant decreasing trend of mean values in parallel with GFR reduction. The prevalence of hypercholesterolemia, smoking, and obesity, as well as the mean values of BMI, showed significant intergroup variations, without a continuing trend though related to GFR values. The prev-

alences of male gender, low HDL, as well as the mean values of diastolic pressure, did not show any significant intergroup variations.

Anemia, proteinuria, and hypoalbuminemia showed a significant rising prevalence trends, inversely dependent upon the degree of reduction of glomerular filtration, i.e. the prevalence of the above variables increased in parallel with the decline of renal function. The levels of hematocrit and serum albumins showed a positive correlation with decreasing GFR, i.e. a significant decrease dependent upon the decreasing renal function. The number of leukocytes in the peripheral blood smear and level of serum fibrinogen did not demonstrate any significant intergroup variations.

Discussion

Risk factor burden, for both traditional and non-traditional factors, is significantly higher among the patients with chronic kidney disease compared to the general population. However, this burden varies significantly with patient characteristics, including the degree of renal dysfunction and cause of renal disease. Even after correction for the factors of gender, age, race, elevated blood pressure, cardiovascular disease, diabetes, and other demographic characteristics, a relatively strong association was still present between the total burden with cardiovascular risk factors and GFR lower than 60 ml/min/1.73m^2 [4,5,9,10].

Within the Modification of Diet in Renal Disease (MDRD) study, Sarnak et al. [11] found out that traditional risk factors were highly prevalent in hronic kidney disease and that their prevalence varied based on the level of renal

Table 2 Cardiovascular risk factors

	total number of examinees 205	> 60 ml/min 60	30 – 59 ml/min 86	15 – 29 ml /min 59
Traditional risk factors				
Age *+	55.1 ± 4.9	52.3 ± 7.7	59.7 ± 8.9	63.0 ±4.5
gender (male) *o	48.8% (100/205)	51.7% (31/60)	47.7% (41/86)	47.5% (28/59)
hypertension % *+	65.4% (134/205)	46.7% (28/60)	67.4% (58/86)	81.4% (48/59)
systolic blood pressure mmHg *+	139 ± 12.6	135 ± 11.9	143 ± 15.3	149 ± 13.8
diastolic blood pressure mmHg *o	85 ± 4.4	84 ± 3.9	86 ± 9.1	87 ± 7.1
obesity % *±	27.3% (56/205)	20.0% (12/60)	31.4% (27/86)	28.8% (17/59)
BMI (kg/m^2) *±	28.0 ± 2.5	26.8 ± 1.8	29.4 ± 2.2	27.9 ± 3.4
glycemia mmol/l *+	6.33 ± 0.74	6.08 ± 0.52	6.54 ± 1.13	7.11 ± 0.96
diabetes % *+	18.5% (38/205)	15.0% (9/60)	17.4% (15/86)	23.7% (14/59)
total cholesterol mmol/l *+	5.82 ± 0.81	5.53 ± 1.20	5.71 ± 0.77	6.26 ± 1.35
hypercholesterolemia % *±	24.9% (51/205)	18.3% (11/60)	29.1% (25/86)	25.4% (15/59)
HDL mmol/l *-	1.25 ± 0.56	1.32 ± 0.47	1.15 ± 0.30	1.00 ± 0.34
low HDL % *o	6.3% (13/205)	6.7% (4/60)	5.8% (5/86)	6.7% (4/59)
triglycerides mmol/l *+	2.18 ± 1.13	1.19 ± 0.60	1.90 ± 0.72	2.94 ± 0.88
hypertriglyceridemia % *+	24.4% (50/205)	18.3% (11/60)	24.4% (21/86)	30.5% (18/59)
hypertrophy LV % *+	22.9% (47/205)	18.3% (11/60)	22.1% (19/86)	28.8% (17/59)
smoking % *±	24.9% (51/205)	31.7% (19/60)	20.9% (18/86)	23.7% (14/59)

Legend: *+ - positive trend
*- - negative trend
*± - inter-group variations without a continuous trend
*o - without trend and without intergroup variations

Table 3 Cardiovascular risk factors II

	total number of the examinees 205	> 60 ml/min 60	30 – 59 ml/min 86	15 – 29 ml/min 59
Non-tradicional risk factors				
proteinuria % *+	20.0% (41/205)	11.3% (8/60)	17.4% (15/86)	30.5% (18/59)
microalbuminuria % *±	28.3% (58/205)	21.7% (13/60)	32.6% (28/86)	28.8% (17/59)
anaemia % *+	25.4% (52/205)	10.0% (6/60)	25.6% (22/86)	40.7% (24/59)
hematocrit % *-	38 ± 3.7	42 ± 5.6	39 ± 4.3	33 ± 2.5
leukocytes 10^9/l *o	6.20 ± 0.89	6.17 ± 0.93	5.98 ± 1.05	6.24 ± 1.44
S - albumin g/l *-	39.0 ± 7.7	40.9 ± 5.8	38.7 ± 6.6	37.2 ± 4.4
S - albumin < 3.5 g/l % *+	7.8% (16/205)	5.0% (3/60)	5.8% (5/86)	13.5% (8/59)
S - fibrinogen g/l *o	3.15 ± 0.54	3.16 ± 0.27	3.03 ± 0.63	3.22 ± 0.48

Legend: *+ - positive trend
*- - negative trend
*± - inter-group variations without a continuous trend
*o - without trend and without inter-group variations

function, that GFR was inversely correlated with blood pressure level and positively correlated with serum HDL cholesterol level, while there were no associations with LDL cholesterol. In our examinees, systolic blood pressure and serum total cholesterol and triglyceride levels rose in parallel with declining GFR. Diastolic blood pressure was without any significant variations related to GFR alterations. There were 65.4% hypertensive individuals among the examinees, with an observed growing trend with decreasing GFR.

Jingers et al. [12] in their prospective study that lasted over 10 years observed an abnormally high incidence of atherosclerotic cardiovascular complications in pre-dialysis patients with CKD in all age groups and in both genders; their total incidence was almost three times higher than that in the general population. Smoking, poor blood pressure control, dyslipidemia with low serum HDL cholesterol, as well as higher degrees of hyperfibrinogenemia and hyperhomocysteinemia were present in CKD patients in whom atherosclerotic cardiovascular complications developed. The same factors favoring atherogenesis in the general population, such as hypertension, smoking or dyslipidemia, were present in CKD patients, but with potentiated effects, so that the incidence and severity of these disorders were higher in uremic patients. Among our examinees, there were 24.9% (51/205) smokers. Smoking is a very important, independent risk factor. Atherogenic effects of smoking in uremic patients are particularly striking due to increased free radical production and consequential lipid peroxidation, nevertheless increased in uremic patients [13].

In the reports by Foley et al. [1,5], individuals with renal failure were older, male, diabetic, with elevated blood pressure, smokers, and physically inactive. Hypertension is in CKD the principal risk factor for cardiovascular atherosclerotic events (especially a high systolic blood pressure). Our examinees were 55.1±4.9 years old on average, being older with declining GFR values. Male gender was more prevalent, with 48.8% (100/205), and the ratio did not change with the progression of chronic renal failure. The average prevalence of diabetes was 18.5% (38/205), with average serum glucose 6.33±0.74 mmol/l. The prevalence of diabetes in group C, with lowest GFR values, was 23.7% (14/59). LVH was present in 22.9% (47/205), according to the echocardiographic parameters, with an incresing prevalence in CKD patients with progressively lower GFR values.

It is well known that lipid parameters are markedly altered in CKD patients compared to the general population, but the changes are more intense in patients with cardiovascular events [3,10,12]. Uremic dyslipidemia, i.e. low HDL cholesterol values, is the major and independent cardiovascular risk factor that occurs early during the course of chronic renal failure. Jungers et al. [12] showed that total and LDL serum cholesterol, apoB and triglyceride levels were significantly higher, while HDL cholesterol was lower, in the group of kidney patients with cardiovascular accidents compared to those without accidents. HDL cholesterol alterations in our examinees were very interesting. Expressed in absolute values, low HDL cholesterol was seen in 6.3% (13/205) of the examinees, and the average serum value of HDL cholesterol was 1.25±0.56 mmol/l, as described in several other studies as well [11,14]. The mean values of serum HDL cholesterol demonstrated a significant declining trend in parallel with declining GFRs, but viewed through the prevalence of patients with low HDL cholesterol values, this risk factor did not demonstrate any significant intergroup differences. The total prevalence of hypercholesterolemia was 24.9% (51/205), being significantly higher in groups B and C compared to group A, with the average serum cholesterol value of 5.82±0.81 mmol/l. Increased triglyceride levels were encountered in 24.4% (50/205), with an average serum triglyceride value of 2.18±1.13 mmol/l. Hypertriglyceridemia was most prevalent in group C, with 30.5% (18/59).

McDonald et al. [15,16] reported a very high rate of pathological albuminuria among the members of a small community of Aboriginal people, the ethnic group with a high incidence of terminal renal failure. Their high levels of albuminuria were associated with low levels of estimated glomerular filtration rate. Albuminuria was also accompanied by increased BMI, elevated blood pressure, elevated HbA1 levels and diabetes. The total proportion of our patients with proteinuria was 20.0% (41/205), with the prevalences of proteinuria and serum hypoalbuminemia on the rise with the progression of chronic renal failure, and inversely dependent upon declining GFR values.

The issues of obesity and chronic renal failure have been investigated in numerous studies, attempting to prove the cause and effect relationship between a rising BMI and declining GFR [17]. In this study, there were 27.3% (56/205) obese examinees, with the average BMI of 28.0±2.5 kg/m^2. Obesity and mean BMI values showed significant intergroup variations and were without any continuing trend related to declining GFR.

The levels of hematocrit and prevalence of anemia in our examinees showed a positive correlation with declining GFR, i.e. a significant declining trend depending upon the declining renal function. The serum level of fibrinogen did now show any significant intergroup variations. In contrast, in the studies by Jungers et al. [12] and Muntner et al. [9] the serum level of fibrinogen was conspicuously higher in CKD patients compared to the healthy individuals, and significantly higher in CKD patients with cardiovascular events compared to the healthy individuals.

The number of leukocytes in the peripheral blood smear did not show any significant intergroup variations. Some studies have reported that leukocytes behave like non-traditional risk factors and as such, they are elevated in CKD [9, 18].

Conclusion

In pre-dialysis CKD patients, a high prevalence of the examined traditional and non-traditional cardiovascular risk factors was established. The cardiovascular risk progressively rises with declining GFR values, and the risk is significantly elavated already in the initial stages of chronic renal failure.

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Original Article

APPENDICITIS IN CHILDREN: SYMPTOMS AND SIGNS, LABORATORY AND HISTOPATHOLOGY FINDINGS IN 67 PATIENTS

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Abstract. *Acute abdominal pain is a reason for hospital admission of about 20% of children. Typical clinical presentation of appendicitis may be significantly different in children. Diagnosis is based on the combination of symptoms, clinical signs, and results of laboratory and radiology examinations. The objective of the present study was to analyze symptoms, signs, laboratory and histopathology findings in children who underwent surgery for acute appendicitis. Sixty-seven patients (37 males and 30 females) with mean age of 9.77 years, operated on for clinical diagnosis of acute appendicitis were enrolled in the study. Abdominal pain was present in all patients, followed by vomitus and fever. Laboratory markers of inflammation varied significantly with severity of inflammation, but were normal in chronic appendicitis. Clinical and histopathology assessments of inflammation were concordant in 22 – 43% depending of the degree of appendicitis. Perforation occurred in 26.86% and negative appendectomy rate was 6%.*

Key words: *appendicitis, children, appendectomy, histopathology.*

Introduction

Acute abdominal pain constitutes 20% of all pediatric surgery admissions [1]. Typical clinical presentation of appendicitis that includes pain, nausea, vomiting and low-grade fever sometimes accompanied by constipation or diarrhea may significantly differ in children[2]. A child may present with very few symptoms and in good general health or in very severe general condition with signs of septic shock and/or bowel obstruction. Decision making is based on the clinical course, physical signs, laboratory and radiology findings. Overall rupture rates varied from 20% to 76%, with a median of 36% in a recent analysis of data from 30 pediatric hospitals in the United States [3]. Perforation rate is reported to be higher in children younger than 5, and is nearly 100% in those younger than 3 years [4,5]. Delay in diagnosis is associated with increased risk of perforation, and it correlates more with pre-hospital than with in-hospital delay [6,7].

Despite the improvement in diagnosis, some of the appendices removed under high clinical suspicion of acute appendicitis turn out to be normal on histopathology (negative appendectomies - NA). Recent researches revealed that some of them had manifested the signs of inflammation on more detailed examination or on immunohistochemistry [8–10].

White blood cell (WBC) count, percentage of neutrophils (Ne%) and C-reactive protein level (CRP) are the most commonly used laboratory markers [11,12], with combined specificity of 95% for acute and 100% for perforated appendicitis [13]. Recently, neutrophil/lymphocyte ratio (NLR) was reported as more reliable marker of acute appendicitis than WBC count [14], and values of NLR higher than 3.5 were strongly associated with acute appendicitis.

Objective of the present study was to analyze symptoms and signs, laboratory and histopathology findings in children who underwent surgery for acute appendicitis.

Materials and Methods

Sixty-seven patients, operated on for clinical diagnosis of acute appendicitis, were enrolled in the study.

Data on patients' demographics, history, symptoms and signs at presentation, physical and laboratory findings were obtained from the medical records. Clinical classification of the degree of inflammation made by treating surgeon has been obtained from operative reports. All removed appendices were routinely sent to histopathology examination. The histopathology classification was based on pathologists' reports. Pathologists were blinded for clinical classification of the degree of inflammation.

Collected data were entered in prepared MS Excel 2007© spread sheet. Statistical analysis was performed using Graph Pad Prism 5© statistical software.

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Results

Mean age of patients was 9.77 ± 2.88 years (range 4.25 – 15.75). There were 37 males and 30 females. Patients' demographics are summarized in Table 1. Forty-one (61%) patients came from urban, and 26 (39%) from the rural areas. Forty-nine (73%) patients were from local community while 18 patients (27%) had been referred from the surrounding secondary level regional general hospitals (RGH). Ten out of 18 referred patients (55%) presented with perforation.

All patients complained of abdominal pain. Symptoms and their duration are summarized in Table 2. Forty-three patients vomited before admission. Thirty-four patients had normal body temperature. After admission, only 10 patients remained afebrile. Seventeen patients presented with constipation and 11 with diarrhea. Table 3 summarizes the presence of the signs of appendicitis.

Mean value of WBC count was 16.24 x 10^3/μL. Mean percentage of neutrophils was 73.79% and average NLR ratio was 6.08. Mean value of CRP was 54.38 mg/ml (Table 4).

Time from admission to operation varied with severity of inflammation (Figure 1). Clinical and histopathology diagnoses, as well as concordance of clinical and histopathology assessment of the degree of inflammation are given in Table 5 and Figure 2. Pediatric surgeon's and pathologist's reports were concordant in 35% of acute, 43% of phlegmonous, 33% of gangrenous and 22% of perforated appendices. Overall concordance in clinical and histopathology reports was 33.34% (21 of 67 patients). According to Cohen's kappa coefficient (Kappa= 0.141), the strength of agreement was considered to be poor.

Table 1 Demographic data of patients operated on with diagnosis of acute appendicitis

Histopathology grade of inflammation	Age (mean± SD)	Gender		Living area		Referral	
		Male	Female	Urban	Rural	Local	RGH
Acute	8.88 ± 2.81	4	6	2	4	6	4
Phlegmonous	9.38 ± 3.09	16	15	19	12	23	8
Gangrenous	10.78 ± 2.52	9	4	8	5	9	4
Perforated	10.80 ± 2.23	4	1	1	4	3	2
Chronic	11.54 ± 1.65	1	3	3	1	4	0
Normal (NA)	8.73 ± 3.56	3	1	4	0	4	0
Total	9.77 ± 2.88	37	30	41	26	49	18

Table 2 Symptoms of appendicitis and its duration

Duration	Symptoms							
	Vomitus		Body temperature					
			Before admission			After admission		
	Yes	No	< 36.9	37.0-37.9	>38.0	< 36.9	37.0-37.9	>38.0
<6h	3	5	6	1	1	1	6	1
6 - 12h	7	1	4	1	3	1	4	3
12 - 24h	23	7	17	9	4	4	17	9
24 - 48 h	6	5	2	2	7	1	3	7
>48h	4	6	5	3	2	3	3	4
Total	43	24	34	16	17	10	33	24

Table 3 Presence of signs of appendicitis

Sign	no	yes	n/a	(%)
Abdominal tenderness	0	67	0	100.0
Rebound tenderness (Blumberg's sign)	16	28	23	41.8
Localized guarding	59	8	0	11.9
Diffuse guarding (defense)	58	9	0	13.4
Diarrhea	53	11	3	16.4
Constipation	47	17	3	25.4

n/a – not applicable (presence or absence of sign was not recorded)

Table 4 Mean values of laboratory markers of inflammation (WBC, % of neutrophils, Ne/Ly ratio and CRP) in study groups and in different grades of appendicitis

Histopathology grade of inflammation	Laboratory marker of inflammation (mean ± SD)			
	WBC count (n x 10^3/μL)	Ne%†	NLR	CRP§ (mg/ml)
Acute	13.55 ± 6.23	67.53 ± 11.92‡	7.1 ± 10.92	35.40 ± 44.07
Phlegmonous	17.36 ± 6.39	78.08 ± 9.74‡,$	6.0 ± 3.20€	49.85 ± 71.68
Gangrenous	16.45 ± 4.03	76.41 ± 18.65	6.8 ± 3.17£	66.41 ± 67.28
Perforated	22.34 ± 7.88	81.60 ± 11.58¥	16.2 ±10.87	66.41 ± 67.28
Chronic	8.175 ± 1.87*	61.45 ± 9.85$,¥	2.2 ± 1.31€,£	13.41 ± 11.98
Normal (NA)	14.00 ± 9.34	68.15 ± 14.91	3.9 ± 2.74	55.86 ± 67.26
Total	16.24 ± 6.59	73.79 ± 10.41	6.1 ± 4.98	54.38 ± 67.45

WBC – White blood cells; Ne% – percent of neutrophils; NLR – Neutrophil to lymphocyte ratio; CRP – C-reactive protein; NA – negative appendectomy. *Mean WBC count was significantly lower in chronic appendicitis ($p<0.05$). †Differences in Ne% among histology groups were significant ($p<0.05$; One-way ANOVA) ‡$p<0.05$, $<0.01; ¥ $p<0.05$.Significant differences in NLR were observed between phlegmonose and chronic € $p<0.05$ and gangrenous and chronic appendicitis £ $p<0.05$. § Differences in mean values of CRP were not significant ($p>0.05$).

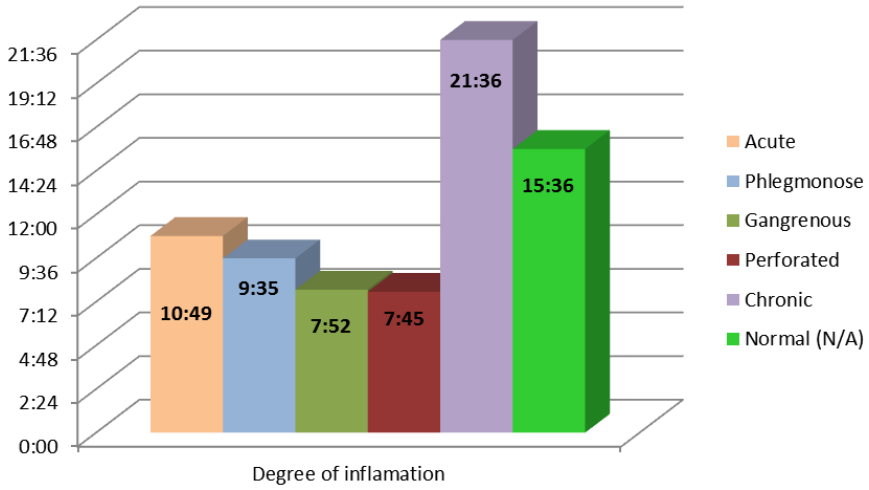

Fig. 1 Time from admission to surgery in relation to intensity of inflammation

Table 5 Distribution of clinical and histopathology degree of inflammation and concordance between clinical and histopathology findings.

Clinical diagnosis			Histopathology diagnosis						Concordant diagnoses	
Degree of inflammation	No	%	Acute	Phlegmonous	Gangrenous	Perforated	Chronic	Normal (N/A)	No	%
Acute	20	29.85	6	9	0	0	1	4	6	30.00
Phlegmonous	14	20.90	4	6	1	0	3	0	6	42.86
Gangrenous	15	22.39	0	9	5	1	0	0	5	33.33
Perforated	18	26.87	0	7	7	4	0	0	4	22.22
Chronic	0	0.00	0	0	0	0	0	0	0	0.00
Normal (N/A)	0	0.00	0	0	0	0	0	0	0	0.00
Total	67	100	10	31	13	5	4	4	21	
%			14.93	46.27	19.40	7.46	5.97	5.97	31.34	

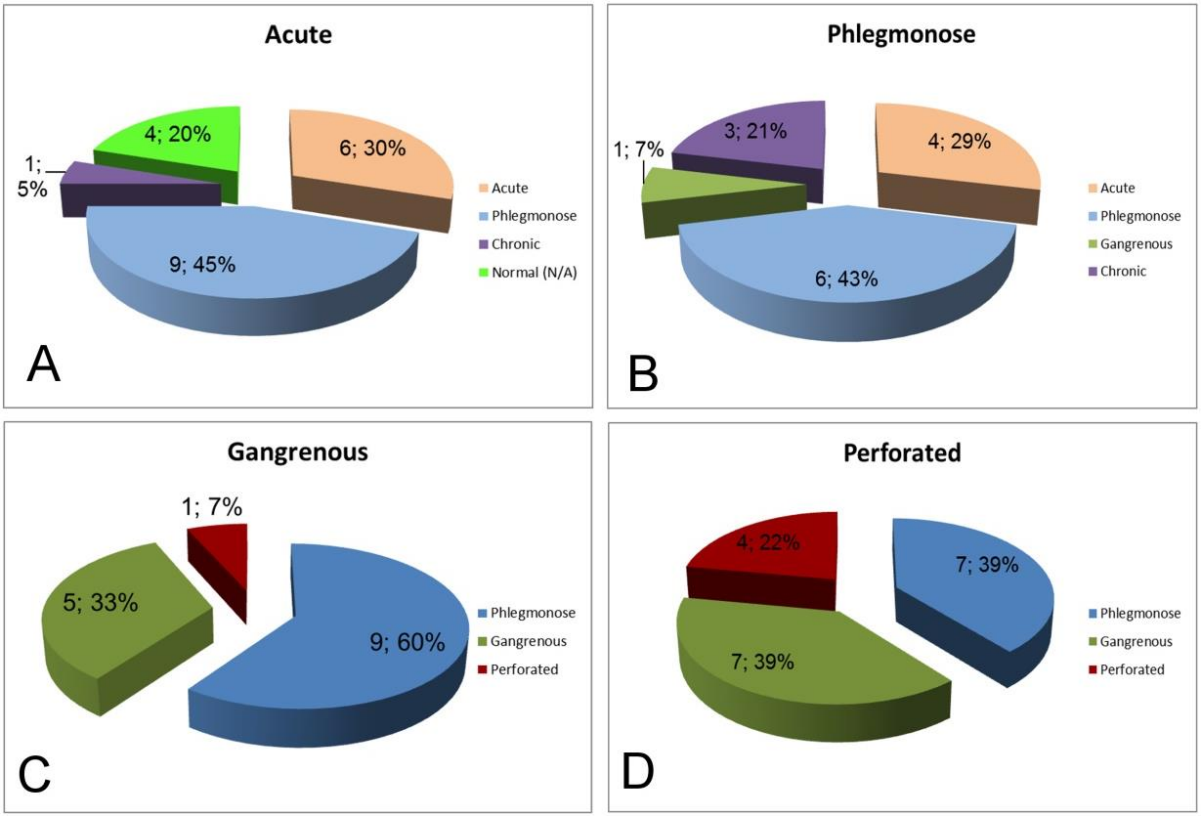

Fig. 2 Histopathology diagnoses in different groups of clinically assessed degree of inflammation and concordance of clinical and histology reports. A – Acute appendicitis, concordance 30% ; B – Phlegmonous appendicitis, concordance 43%; C – Gangrenous appendicitis, concordance 33%; D – Perforated appendicitis, concordance 22%.

Discussion

Appendicitis is the most common reason for emergency abdominal surgery in children [15] with peak incidence in the second and third decade of life. Average age of patients in our study was 9.77 years. Appendicitis is rare in children younger than 5 and most of them present with perforation. The reasons for low incidence of appendicitis in young children are not clear, but distinct anatomical, pathophysiological and social characteristics of children contribute to high perforation rate in children younger than 5 years. Four of our patients (5.97%) were in that age group and half of them had perforations. Slight male preponderance was observed (male: female ratio 1.23), with the highest predominance of boys in group of perforated appendicitis (72%).

Symptoms and signs

Abdominal pain was a universally present symptom. Most frequently, it was located in the right lower quadrant. Description of its migratory nature had not been easily obtained, particularly in younger children. Common duration of pain was 12-24h before admission. Very short duration of pain, less than 6 hours, was associated with more severe inflammation, while duration of pain more than 48h corresponded to either acute or perforated appendicitis. These findings slightly differ from those of Rothrock and Pagane [16]. Recent suggestions that acute appendicitis in fact may be the result of repeated episodes of inflammation and that acute and perforated appendicitis might be different entities may support our findings [6,17]. Vomiting had low sensitivity and specificity in limited number of reports on pediatric patients [18,19]. Two-thirds of our patients vomited. Most of the patients with 3-5 episodes of vomitus had ruptured or gangrenous appendicitis. Less than 2 or more than 5 episodes of vomitus corresponded with acute inflammation.

O'Shea et al. [20] reported sensitivity of 0.75 and specificity 0.78 for fever as a symptom of appendicitis, while Andersson et al. [21] found that body temperature provided important diagnostic information, particularly in advanced appendicitis. Normal body temperature from onset of symptoms to admission was present in 52% of our patients. Nevertheless, 85% of them had fever after admission. Ten patients (15%) remained afebrile during hospital course but histopathology showed only one normal appendix (NA).

All children in our series had abdominal tenderness in right lower quadrant (RLQ). Rebound tenderness (Blumberg's sign) was positive in 28 patients (41.79%). In adults, rebound tenderness is the single most accurate physical sign, with accuracy of 86% [18], but its accu-

racy decreases with age to 43% in pediatric patients [18,21]. Localized guarding in RLQ was present in 8 children (11.94%) and 6 of them had clinically perforated appendicitis.

Laboratory markers of inflammation

Mean values of WBC were elevated in patients with acute inflammation and in negative appendectomies but were normal in children with chronic appendicitis ($p<0.05$; one-way ANOVA; Table 4). Values of Ne% were significantly different among histology groups ($p= 0.05$; one-way ANOVA; Table 4). Mean values of Ne% were statistically different between acute and phlegmonous ($p<0.05$), phlegmonous and chronic ($p<0.01$) and perforated and chronic ($p <0.05$) appendicitis. Differences in NLR were statistically different between groups with phlegmonous and chronic ($p<0.05$) and gangrenous and chronic ($p<0.05$) inflammation. Differences in values of CRP were not significant ($p>0.05$; one-way ANOVA; Table 4) Three out of 4 patients with chronic appendicitis had normal WBC, Ne% and NLR, but only one had normal CRP. Among children with negative appendectomy, all inflammatory markers were normal in 2 children and 2 had high levels of WBC and CRP. Results of Siddique et al. [13] as well as our findings, demonstrated that mean values of WBC and CRP increase with severity of inflammation. However, both markers are general markers of inflammation, not specific for appendicitis. Nevertheless, appendicitis cannot be ruled out solely on the basis of normal laboratory findings, in the presence of other suggestive clinical signs.

Accurate assessment of the degree of inflammation has implication on postoperative therapy protocol and complication rate. Overall reported accuracy of macroscopic assessment of the appendix at surgery was 87.3% [22] with 100% consensus for perforation, abscess, or gangrene of the appendix. Conversely, Bliss et al. [23] reported greater agreement of surgeon's and pathologist's assessment for normal and acutely inflamed appendices than for more advanced forms. In our study, pediatric surgeons tend to underestimate acute and overestimate more advanced forms of appendicitis (Figure 2). Clinical diagnosis of perforation was established in 26.86% of patients, with histopathology confirmation in 7.46% (22% of concordance). The remaining 14 clinically perforated appendices were histologically either gangrenous or phlegmonous (39% each). Surgeons tend to classify appendix as perforated in the presence of turbid fluid in abdomen, even if the actual hole in appendix wall has not been visualized. On the other hand, pathologists may omit small holes particularly if the whole appendix has not been examined. Strict definition of perforation as a hole in appendix or free appendicolith in abdomen may improve concordance of surgeon's and pathologist's reports in advanced forms of appendicitis [24]. Diagnosis of chronic appendicitis and negative appendectomy could not be established without histopathology.

Time from admission to operation varied with the severity of inflammation from average of 10h 49 min for acute to 7h 45 min for perforated appendicitis. Patients with negative appendectomies were operated on 15h 36 min and those with chronic inflammation 21h 36 min after admission. Narsule et al. [6] reported similar results. In patients with milder or equivocal symptoms and signs repeated examinations, laboratory tests and imaging studies may be necessary. On the other hand, children who were septic, dehydrated and with signs of generalized peritonitis on admission, may benefit from short period of IV fluids and antibiotic administration before operation, but this may result in intra-hospital delay of surgery. Yardeni at al. [25] reported that overnight delay of surgery in those patients who presented with non-perforated appendicitis did not significantly affect operating time, rate of perforations or frequency of complications.

Negative appendectomy rate of 6% in our study is low and comparable with other reports [6,26]. Likewise, clinical perforation rate of 26.86% is similar to other reports [6]. It is biased to some extent with the fact that more than half of children with perforation (10 of 18) was referred from other RGH. The main reason for referral was severe clinical presentation. Six referred patients with perforation of the appendix had clinical signs of acute abdomen and x-ray findings of intestinal obstruction. None of the referred patients were treated in RGH before referral. Perforation was found in 3 patients admitted less than 6 hours after onset of the symptoms. Two of them were operated on within 3 hours after admission. These results may support theories [17] that two distinct populations of patients with appendicitis may exists, and that in some patients, inflammation progresses more rapidly to perforation [6]. Furthermore, this implies that perforation correlates more with prehospital than with in-hospital delay, and that postponing operation for several hours in order to clarify diagnosis in equivocal cases would not increase the risk of perforation [25,27].

In conclusion, clinical presentation of appendicitis in children may vary, from those with very mild symptoms to those with life threatening septic shock and bowel obstruction. Unfortunately, there is neither a single diagnostic test nor a combination of clinical, laboratory, and imaging studies, that have 100% of sensitivity and/or specificity. Furthermore, there is still no way to distinguish simple acute and perforated appendicitis with certainty before surgery. Delaying surgery for several hours in doubtful cases to clarify diagnosis may further reduce negative appendectomy rates without increasing the risk of perforation.

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Original Article

INITIAL EXPERIENCES IN TREATMENT OF GASTROINTESTINAL FOREIGN BODIES IN CHILDREN

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Abstract. *We performed a retrospective analysis of all records of children with ingested foreign bodies presented to Clinical Center of Niš Pediatric Clinic and Pediatric Surgery and Orthopedics Clinic in the period from January 2014 to June 2017. The most commonly detected foreign bodies were: metal coins (7) followed by hairclips (2), metal key (1), trichobezoar (1) magnets (1) button battery (1) and zipper puller (1). Regarding anatomical location, foreign bodies were most frequently found in stomach (in 11 patients) followed by esophagus (in 2 patients) and jejunum (in 1 patient). In the majority of our patients (7) foreign bodies passed out of gastrointestinal tract spontaneously. Endoscopic foreign body removal was performed in 5 cases while surgery as a sole therapeutic action was done in 1 patient. In one child multiple magnets were removed from the stomach performing both endoscopic and surgical interventions. Teamwork of a gastroenterologist and a surgeon is crucial for optimizing therapeutic options for each individual patient. Public awareness of this problem and education of parents should be increased to a higher level in order to prevent cases of foreign bodies ingestion in children.*

Key words: *foreign body, endoscopic, removal, surgery, experience*.

Introduction

In most cases (98%) foreign body (FB) ingestion in children occurs accidentally. The peak incidence is in children between the ages of 5 months and 5 years because during this period, children try to recognize objects from their surroundings by putting them in their mouths. On the other hand, FB ingestion in adolescents is always intentional and raises suspicion of psychiatric illness and substance abuse [1,2]. FBs most commonly found in the digestive tract are coins, batteries, magnets and toy parts. Approximately 80%–90% of ingested FBs pass out of the body spontaneously while the rest 10%–20% may require endoscopic intervention or rarely surgery [3]. Children can be asymptomatic or can present symptoms such as the following: dysphagia, vomiting, drooling, refusal of meals, stridor, and respiratory distress. These patients are prone to various complications that range from negligible to life-threatening [4-6].

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Material and Methods

The records of all children presented to Clinical Centre of Niš Pediatric Clinic and Pediatric Surgery and Orthopedics Clinic for FB ingestion from January 2014 to June 2017 were evaluated retrospectively. The following data were analyzed: gender and age of patients, presence of symptoms, foreign bodies type, size and location, management, complications and outcome. Radiography of the neck, chest and abdomen were done in order to determine FB location.

Endoscopic FB removal under general anesthesia was the most frequent procedure performed in our patients. Surgical interventions were done in cases when endoscopy was inefficient. All upper digestive endoscopies were done by gastroscope Olympus GIF Q180. Endoscopic tools such as: rat-tooth and alligator graspers, as well as diathermic snare were used in endoscopic FB removal.

Results

Fourteen patients (8 girls, 6 boys) at the age from 8 months to 8 years were found to have FBs in digestive tract. Presenting symptoms were: vomiting (in 2 patients), drooling (in 1 patient) refusal of food (in 1 patient) and dysphagia (in 1 patient), while 9 patients were asymptomatic. In 6 cases parents witnessed FB ingestion in their children.

The most commonly detected types of FBs were metal coins (7), followed by hairclips (2), metal key (1), trichobezoar (1), magnet (1), button battery (1) and zipper puller (1) (Tab. 1). Regarding anatomical location, FBs were most commonly found in stomach (in 11 patients) and esophagus (in 2 patients) followed by jejunum (in 1 patient). In all patients, except one with trichobezoar, the FB position were determined by radiography.

In 7 children, FBs passed out of digestive tract spontaneously. Endoscopic removal was done in 5 cases. The types of removed FBs were: coins (in 3 cases) and hairclips (in 2 cases). In one patient multiple magnets were removed from stomach performing both endoscopic and surgical interventions. Surgical removal as the sole intervention was done in 1 patient due to gastric trichobezoar.

Mucosal ulcerations and erosions as a consequence of FB impaction were observed in 3 cases (hairclip in the esophagus, hairclip in the stomach, magnets in the stomach). In one patient, (8-month-old girl) during endoscopic removal a long hairclip was stuck in the hypopharynx. The hairclip was successfully pulled out using a pair of Magill forceps.

Table 1 Clinical characteristics of children with gastrointestinal foreign bodies

Patient's number	Patient's sex and age	FB type	Location	Outcome
1.	F, 8 m	Hairclip	Esophagus	ER
2.	M, 4 y	Coin	Esophagus	ER
3.	M, 2 y	Coin	Stomach	ER
4.	F, 4 y	Coin	Stomach	ER
5.	F, 18 m	Coin	Stomach	PS
6.	M, 22 m	Coin	Stomach	PS
7.	F, 5 y	Coin	Stomach	PS
8.	M, 3 y	Coin	Stomach	PS
9.	F, 4 y	Magnets	Stomach	ER+SR
10.	F, 5 y	Hairclip	Stomach	ER
11.	M, 3 y	Zipper puller	Stomach	PS
12.	M, 8 y	Trichobezoar	Stomach	SR
13.	F, 3y	Metal kee	Stomach	PS
14.	F, 6 y	Button battery	Jejunum	PS

F – female, M – male, m – months , y – years
FB – foreign body, ER – Endoscopically removed, SR – surgically removed, PS – passed spontaneously

Table 2 Timing of endoscopic intervention in pediatric foreign body ingestions

Type	Location	Symptoms	Timing
Button battery	Esophagus	Yes or No	Emergent
	Gastric/SB	Yes	Emergent
		No	Urgent (if age <5 and BB ≥20 mm)
			Elective (if not moving on serial x-ray)
Magnets	Esophagus	Yes	Emergent (if not managing secretions, otherwise urgent)
		No	Urgent
	Gastric/SB	Yes	Emergent
		No	Urgent
Sharp	Esophagus	Yes	Emergent (if not managing secretions, otherwise urgent)
		No	Urgent
	Gastric/SB	Yes	Emergent (if signs of perforation, then with surgery)
		No	Urgent
Food impaction	Esophagus	Yes	Emergent (if not managing secretions, otherwise urgent)
		No	Urgent
Coin	Esophagus	Yes	Emergent (if not managing secretions, otherwise urgent)
		No	Urgent
	Gastric/SB	Yes	Urgent
		No	Elective
Long object	Esophagus	Yes or no	Urgent
	Gastric/SB	Yes or no	Urgent
Absorptive object	Esophagus	Yes	Emergent (if not managing secretions, otherwise urgent)
		No	Urgent
	Gastric/SB	Yes or no	Urgent

BB – button battery; SB – small bowel.
Kramer RE, et al. Management of ingested foreign bodies in children: a clinical report of the NASPGHAN endoscopy committee. J Pediatr Gastroenterol Nutr 2015; 60: 562-574

Discussion

Every child suspected to have ingested a FB should have the frontal and lateral radiography of the head, neck, thorax and abdomen done in order to determine the foreign body kind and location within the digestive tract. It should be kept in mind that plastic and wooden FBs, as well as the animal bones may not be seen in the radiograph. When ingestion of these FBs is suspected upper digestive endoscopy should be done.

Flexibile endoscopy under endotracheal general anesthesia is a method of choice for extraction of FBs from the digestive tract [7]. The rigid endoscopy is an alternative method in cases of sharp FBs presence in the hypopharinx and the proximal esophagus. Endoscopy should be done by a skillful and experienced endoscopist, who should fuse all kinds of endoscopic tools for extraction of FBs, such as: rat-tooth and alligator forceps, polypectomy snare, retrieval net, biopsy forceps, etc. According to the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition endoscopy committee's recently revised guidelines for management of ingested FBs in children, the timing of the FB removal depends on the FB type, location, the presence of symptoms, and the time of the patient's last oral intake and can be: emergent [< 2 hours from presentation, regardless of nil per os (NPO) status], urgent [< 24 hours from presentation, following usual NPO guidelines] or elective [>24 hours from presentation, following usual NPO guidelines] (Tab. 2) [7].

Surgery may be one of the alternative methods, particularly in cases when it is not possible to extract the FB by means of endoscopy (trichobezoar, greater number of magnets, sharp foreign bodies inaccessible by endoscopic examination and causing symptomatology) [8]. Magill forceps may be used for extraction of FBs from oropharynx and the upper 1/3 of esophagus [9,10].

All our patients except the one with trichobezoar in his stomach, had the FBs positioned in the digestive tract as determined on radiographs (Fig. 1). The FB most frequently found in the digestive tract, was a metal coin (in 7 cases) (Tab.1). Coins, as most frequently found FBs in the digestive tract, have also been mentioned in studies with a great number of children with ingested foreign bodies [11-13]. From the total number of our 14 patients with ingested FBs, spontaneous elimination of a FBs from gastrointestinal tract occurred in 7 cases. Indication for emergent endoscopic extraction existed in 2 cases: in one patient with a hairclip in esophagus and in one with a coin in the esophagus (Fig. 2a,b).

We have chosen to use endoscopic extraction in 4 cases when FBs were retained in the stomach. In 2 cases it was a metal coin, in 1 a hairclip (Fig. 2c) and in one magnets. We decided to do the endoscopic extraction after the coins had been present in the stomach for more than two weeks and had not passed into more distal parts of gastrointestinal tract.

The radiograph of one patient showed a metal object of unknown origin located in the stomach (Fig.1c). In esophagogastroduodenoscopy the metal object was

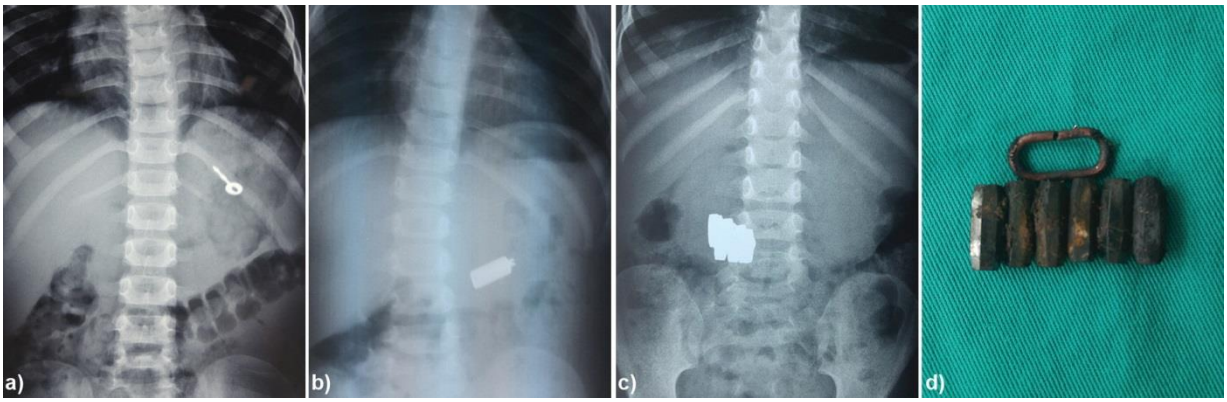

Fig. 1 Gastrointestinal foreign bodies on radiographs and after removal: a) metal key in stomach, b) zipper puller in stomach, c) magnets in stomach, d) magnets and ring after endoscopic and surgical removal

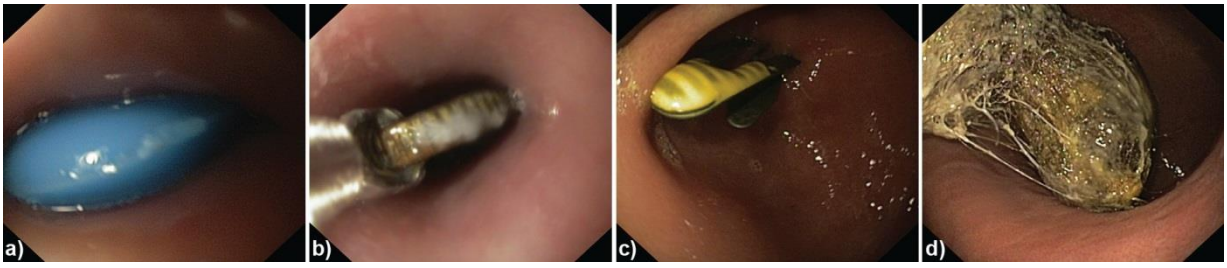

Fig. 2 Endoscopic appearance of gastrointestinal foreign bodies: a) hairclip in esophagus, b) coin in esophagus grasped with rat-tooth forceps, c) hairclip retained in pylorus, d) large gastric trichobezoar

identified as a group of 6 magnets and 1 ring (probably a part of a toy) which mutually covered the stomach antrum mucosa. Two magnets and the ring were removed by endoscopy with the application of polypectomy snare. The remaining four magnets were removed by surgery (Fig. 1d).

In one of our patients, large trichobezoar (Fig. 2d) was removed from the stomach by surgical intervention. Surgical solution for gastric trichobezoar in children has been mentioned in numerous papers as a method of choice [8,14-16].

Radiograph of one child showed a button battery in the jejunum which fortunately passed the digestive tract without any complications. Button batteries can cause current stream generation that may result in tissue necrosis and perforation [17]. Patients with button batteries in esophagus (regardless of being symptomatic or asymptomatic) and symptomatic patients with button batteries in stomach or small bowel represent a medical emergency [7].

Complications due to the FB presence in digestive tract may be various: bleeding, perforations, pneumomediastinum, aspiration pneumonia, etc. The appearance of such complications is directly proportional to FB sharpness and the impaction time [18]. Regarding complications arising because of the very presence of the FB in digestive tract, in our group of patients there were asymptomatic lesions of esophagus and stomach mucosa in three patients. In one patient, there was a complication in the course of the FB (hairclip) removal from esophagus. On that occasion, the hairclip got stuck in the hypopharynx and could not be pulled out even after the patient's position changed or by applying various endoscopic maneuvers. Finally, the hairclip was grasped and pulled out by using Magill forceps.

Conclusion

Our initial experiences in the treatment of gastrointestinal FBs in children confirm without any doubt the significance of the upper digestive endoscopy as a safe and reliable procedure for removing FBs in children. Teamwork of a pediatric gastroenterologist and a surgeon is indispensable in order to respond to all the challenges this problem brings about in pediatric population. Public awareness of this problem and education of parents should be increased to a higher level in order to prevent cases of FBs ingestion in children.

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Original Article

HYSTEROSCOPY BEFORE IN VITRO FERTILIZATION

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Abstract. *In the last decade, success after in vitro fertilization process (IVF) has remained at a similar rate despite all the improvements implemented in the stimulation protocols and laboratory techniques. Hysteroscopy is a method becoming more widely used with patients after a failed IVF cycle, considering a large incidence of uterus cavum pathological states which have a negative impact on the favorable outcome. Numerous studies have provided different results on the IVF outcome with hysteroscopy performed prior to this treatment in cases with no uterus cavum pathology. The aim of the research was to examine the effect of both diagnostic and surgical hysteroscopy on the outcome of IVF. Hysteroscopy was performed with 74 patients 30 to 50 days prior to IVF and in 33 of them (group I) some pathological state was noticed, which was treated during the same procedure. The control group (group III) included 151 patients who had IVF performed with no prior hysteroscopy. There is no statistically significant difference in the rate of post hysteroscopy implantation between I and II group when compared to the control group (20.62% vs 23.28% vs 17.31%), nor in the rate of clinical pregnancies (45.45% vs 46.34% vs 34.44%). Following the correctional treatment of uterus cavum pathological states, implantation and pregnancy rates remain at a level comparable to hysteroscopically normal medical findings. Statistically significant higher pregnancy rate is present in group I after the first IVF cycle, compared to the next IVF in the same group and in comparison to the next IVF cycle in the control group (60.00% vs 27.91%, $p<0.05$). Hysteroscopy is a simple and safe method allowing nearly identical rate of clinical pregnancies after a surgical treatment of uterus cavum pathological states when compared to the control group, but statistically much higher pregnancy rate if the order of IVF procedure is being compared. In cases of normal ultrasound findings and negative hysteroscopical findings, performing hysteroscopy prior to IVF does not provide significantly better results. Therefore, its routine execution is not recommended.*

Key words: *hysteroscopy, pathological states of the cavum, IVF outcome*.

Introduction

In the last decade, pregnancy rate after IVF has remained almost the same, regardless of a lot of improvements implemented in the stimulation protocols and the progress of the laboratory techniques. That being a major reason, more attention has been paid to the removal of all the pathological states which could possibly have a negative impact on the endometrium receptivity, thus lowering the pregnancy rate after IVF [1,2]. Hysteroscopy is a superior method in uterus cavum visualization enabling a surgical treatment of the pathological changes within the same act. A remarkable progress has been made with the inclusion of cameras, an optical system and a small sized hysteroscope itself, avoiding thus a cervical dilatation, alleviating pain during the intervention and enabling it to be performed in outpatient conditions, without using anesthesia – *office hysteroscopy* [3]. It is widely accepted that hysteroscopy is performed after failed IVF cycles. However, there is still no agreed consensus on a required hysteroscopy prior to IVF cycle, especially in cases of normal cavum ultrasound findings. Numerous studies provide different data on IVF success when hysteroscopy is performed immediately prior to the IVF procedure [4–7].

For all these reasons, the aim of this research was to examine the influence of hysteroscopy on the IVF outcome, both in cases with existing pathological substratum in the uterus cavum and in cases with normal hysteroscopic findings.

Methods

The research was done at the Clinic of Gynecology and Obstetrics of the Clinical Center in Niš, as a prospective study, and it included 225 patients from the National IVF program.

Criteria for inclusion in the research were: less than 40 years of age, FSH < 15 IU/ml, AMH > 0.5, body mass index (BMI) < 30 kg/m², lack of genital infection and favorable karyotype of both partners.

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Criteria for exclusion were: presence of chronic systematic disease, existence of hepatitis C or HIV infection, organic pathology of the ovaries, the cause of immune infertility and azoospermia.

The patients were divided in three groups: group of 33 patients who had hysteroscopy performed prior to IVF, with existing uterus cavum pathology treated within the same procedure; II group of 41 patients, with hysteroscopy prior to IVF but with normal hysteroscopic findings; and the III control group of 151 patients with no hysteroscopy.

Hysteroscopy was performed during oral contraceptive therapy, 30–50 days before IVF. Saline solution was used as a distension medium and a 5mm *Bettocchi office* hysteroscope (Karl Storz GmbH and co, Tuttlingen, Germany) with a 5Fr working channel. The patients were in a lithotomy position all with short termed intravenous anesthesia applied. Vaginoscopic approach was used with no ecarter and no cervix traction. Versapoint and Springle electrode (Johnson) were used in cases of polypectomy, septum resections and smaller myomas. In one case only, with a wider myoma, a bipolar resectoscope was used with a 9mm outer cover. Polyps, myomas and unclear lesions were hystopathologically evaluated.

IVF procedure was performed 1–2 months post oral contraceptive therapy. Long and short protocol with GnRH agonists was used. Serial ultrasound checkups during controlled ovary hyperstimulation (COH) were done with a "Shimatzu" ultrasound device, starting with the 6^{th} day of stimulation. On finding 2 or more follicles larger than 18mm, patients got 10000 IU Pregnyl injection, and 34–36 hours afterwards, a transvaginal oocytes pick-up was performed (OPU). Embryo transfer (ET) was done on the 2^{nd}, 3^{rd} or 5^{th} day after the aspiration, monitored by an ultrasound, putting back 3 embryos at the most. "Cook's" catheter was used for the ET. The same therapy was applied for all patients, after ET: Utrogestan 200mg tablets, 3 times a day; Cardiopirin 100mg tablets, once a day; Dexason 0.5mg tabl., once a day. ßHCG from blood was determined for biochemical verification of pregnancy, 15 days post OPU. Clinical pregnancies were verified by transvaginal ultrasound check up, by visualization of the embryo's cardiac activity, 4–5 weeks after ET.

This prospective clinical trial was approved by the Ethics Committee. The treatment of the patients included hysteroscopy and a long and short GnRH agonist protocol. Written informed consent was provided from all the patients participating in the study.

The data were processed by standard descriptive statistical methods (average value, percentage representation). The statistical processing was done among defined groups. Continual variables relative to data distribution were compared using Student's t-test, Pearson's χ^2 test or ANOVA test.

Results

The patients from the examined groups were not significantly different in any of the generally examined parameters (Table 1).

There are also no statistically significant differences among the groups considering: the number of oocytes, conceived embryos, transferred embryos, and the day of embryo transfer as well (Table 2). The long protocol with agonists was most frequently used in the control group when compared to the I group, at a statistical level of significance $p<0.05$. Based on the general parameters and the features of the IVF cycle, homogeneity of the groups is present, which makes further results valid for this research.

Presence of pathological states in the group of patients with hysteroscopy was 44.59% (Table 3), with endometrial polyp as the most common pathological state (63.64%), shown in Graph 1. There is no statistically significant difference in the incidence of uterus cavum pathological states between the first and the next IVF (Table 4).

Although the implantation rate is higher in hysteroscopic groups 1 and 2, compared to the control group (20.62% vs 23.28% vs 17.31%), and the pregnancy rate as well (45.45% vs 46.34% vs 34.44%), there are no significant differences between the groups. There is no statistically important difference neither in the multiple pregnancy rate nor in the rate of biochemical pregnancy, comparing all the three groups (Table 5).

Table 1 General parameters of patients in examined groups

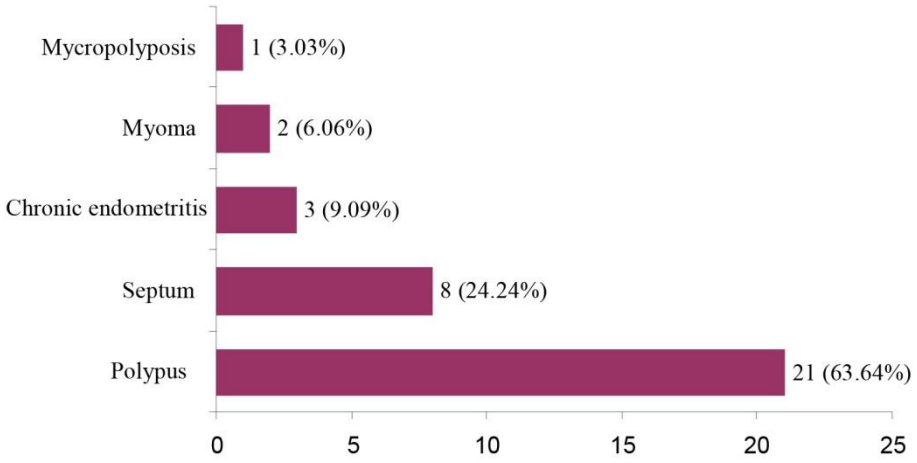
	I group (n = 33)	II group (n = 41)	III – Control group (n = 151)
Age (years)	34.00 ± 2.97 (33.00)	34.00 ± 3.49 (35.00)	33.61 ± 3.65 (34.00)
Patients per age group			
≤30 years	3 (09.09%)	7 (17.07%)	34 (22.52%)
31–35 years	18 (54.55%)	19 (46.34%)	61 (40.40%)
36–40 years	12 (36.36%)	15 (36.59%)	56 (37.09%)
Duration of infertility (years)	7.12 ± 3.53 (6.00)	5.93 ± 2.86 (6.00)	6.38 ± 3.58 (6.00)
FSH (mIU/mL)	6.91 ± 3.10 (6.45)	6.88 ± 2.90 (6.10)	5.93 ± 2.59 (5.50)
AMH (ng/ml)	3.31 ± 3.08 (2.32)	2.92 ± 2.88 (1.84)	3.02 ± 2.47 (2.18)
BMI (kg/m²)	23.85 ± 2.60 (24.00)	23.59 ± 2.96 (23.00)	23.50 ± 2.95 (23.00)

Data are given as absolute numbers (percentages), means ± SD (medians)

Table 2 The IVF cycle features of patients in the examined groups.

	I group	II group	III – Control group
Gonadotropin (IU)	2081.24 ± 804.63 (1850.00)	2078.66 ± 710.05 (1975.00)	2019.97 ± 674.64 (1950.00)
No. of oocytes	9.55 ± 5.08 (9.00)	10.73 ± 7.37 (10.00)	9.98 ± 6.58 (8.00)
No. of embryos	5.73 ± 3.46 (5.00)	5.59 ± 3.54 (5.00)	4.89 ± 3.22 (4.00)
No. of transferred embryos	2.94 ± 0.83 (3.00)	2.58 ± 0.41 (3.00)	2.68 ± 0.20 (3.00)
Protocol			
Long agonists	16 (48.49%)	27 (65.85%)	98 [a*] (64.90%)
Short agonists	17 (51.51%)	14 (34.15%)	53 (35.10%)
Endometrial thickness (mm)	10.23 ± 1.81 (10.00)	9.98 ± 1.57 (10.00)	10.02 ± 1.72 (10.00)
First / second IVF	20 (60.60%) / 13 (39.40%)	26 (63.41%) / 15 (36.59%)	108 (71.52%) / 43 (28.48%)
ET day	3.83 ± 1.03 (3.00)	3.34 ± 0.94 (3.00)	3.65 ± 0.98 (3.00)
2^{nd} day (%)	0 (0.00%)	4 (9.76%)	4 (2.65%)
3^{rd} day (%)	21 (63.64%)	28 (68.29%)	96 (63.58%)
5^{th} day (%)	12 (36.36%)	9 (21.95%)	51 (33.77%)

Data are given as absolute numbers (percentages), means ± SD (medians)
* – p<0.05, a – vs I group



Graph 1. Percentage of presence of pathological findings in I group

Table 3 Presence of pathological findings in the study group

Group	Patient nb	Total %
I + II group	74	100,00%
I group	33	44.59%
II group	41	55.41%

Table 4 Presence of pathological findings in I group related to the first and second IVF

	First IVF n=20		Second IVF n=13	
Polypus	14	70.00%	7	53.85%
Septum	5	25.00%	3	23.08%
Chronic endometritis	1	5.00%	2	15.38%
Myoma	1	5.00%	1	7.69%
Mycropolyposis	0	0.00%	1	7.69%

Table 5 IVF features of patients in the examined groups

	I group		II group		III – Control group	
Implantation rate	20.62%	20 / 97	23.28%	27 / 116	17.31%	72 / 416
Clinical pregnancy rate	45.45%	15 / 33	46.34%	19 / 41	34.44%	52 / 151
Biochemical pregnancy rate	12.12%	4 / 33	12.20%	5 / 41	6.62%	10 / 151
Multiple pregnancy rate	33.33%	5 / 15	36.84%	7 / 19	36.54%	19 / 52

Data are given as absolute numbers (percentages)

Table 6 Pregnancy rate in the examined groups, with first and second IVF cycle compared.

	First VTO			Second VTO		
Group	Patient nb/	Pregnancy nb	% pregnancy	Patient nb/	Pregnancy nb	% pregnancy
I group	20	12*cd	60.00%	13	3	23.08%
II group	26	13	50.00%	15	6	40.00%
III–Control group	108	40	37.04%	43	12	27.91%
Total	158	65	41.14%	74	27	36.49%

Data are given as absolute numbers (percentages)
* – $p<0.05$, c – vs control, d – vs second IVF

Significantly higher pregnancy rate is present in I group after the first IVF cycle in comparison to the second IVF in the same group (60.00% vs 23.08%, $p<0.05$), and also between the first IVF cycle in I group compared to the second IVF in the control group (60.00%vs 27.91%, $p<0.05$) (Table 6).

After hysteroscopic polypectomy, highest pregnancy rate was achieved in comparison to uterus cavum pathological states. However, a number of patients did not allow statistical verification (Table 7).

Table 7 Pregnancy rate in relation to the pathological findings

Hysteroscopic findings	Patient nb/	Pregnancy nb	% pregnancy
Polypus	21 /	12	57.14%
Septum	8 /	3	37.50%
Myoma	2 /	0	0.00%
Chronic endometritis	3 /	1	33.33%
Mycropolyposis	1 /	0	0.00%

Discussion

Regardless of the quality of embryos, an appropriate endometrial thickness and a successful embryo transfer (ET), unsuccessful implantation remains a major cause of IVF method failure. Repetitive implantation failure (RIF) is defined as no conception after two or more alternate transfers of one or more adequate quality embryos. All uterus cavum pathological states have a negative impact on the implantation rate. Their diagnostics is one of the primary goals before entering the IVF cycle.

Our research showed cavum pathology incidence of 44.59% with the largest presence of endometrial polyps, in nearly 2/3 of the cases (63.64%). Fatemi [1] found the frequency of only 11% of uterus pathology, whereas Cenksoy [8] discovered pathological states in 44.9% of the patients. In those two studies, endometrial polyp was the most common, but still in a smaller percentage than in our research, 6- 19.7%. In his journal article, Kodaman [9] discovers a post IVF conceiving benefit from hysteroscopic polypectomy, explaining that an endometrial injury during the intervention is a reason of increased endometrial receptivity. Polyp treatment options are: cancellation of the cycle and polypectomy, polypectomy and freezing of embryos with ET after a few months' period, or ignoring the polyps and continuation of the cycle [10].

Even though the implantation and pregnancy rates after cavum pathology treatment are higher than in the control group, our research did not lead to any statistically significant differences. Comparing the IVF outcomes in the first group of patients with clear hysteroscopic findings, similar percentage can be seen. That is in accordance with numerous studies proving the benefit of performing hysteroscopy prior to IVF with patients having pathological substratum and specifying better IVF results after polypectomy, myomectomy or septum uterus resection [7,11,12].

The influence of hysteroscopy itself on the IVF outcome has been considered for quite a long time, advocating hysteroscopy prior to every IVF cycle. In favor of that, many projects discuss the impact of local endometrial injury during hysteroscopy which brings about endometrial inflammation, thus increasing the endometrial receptivity [13].

In their meta-analyses, Pundir and El-Toukhy found the proof of the hysteroscopy benefit prior to the first IVF cycle, proving a higher pregnancy rate with women who underwent hysteroscopy procedure [4]. One more research with 480 patients also showed higher pregnancy rates when hysteroscopy was performed before the first IVF [14]. Our study did not provide information about hysteroscopy itself influencing statistically better IVF outcome results. Pregnancy rate in II group is statistically not different from the pregnancy rates neither in I group nor in the control group (46.34% vs 45.45% vs 34.44%).

Latest randomized multicentral studies showed that if the ultrasonography result is normal, there is no increased pregnancy rate after hysteroscopy. They also showed that there is no significant difference even if hysteroscopy is being performed after repetitive failed IVF cycles, unless there are pathological changes in the uterus cavum [5,6].

Conclusion

Hysteroscopy is a safe method enabling reliable diagnostics of all uterus cavum pathological states which have a negative impact on the IVF outcome. It allows a simultaneous surgical treatment of the pathological changes within the same procedure and it gives a similar IVF outcome as with patients who have clear cavum

findings. In case of normal ultrasonography findings, there are no statistically better results in the IVF procedure after hysteroscopy. Therefore, routine hysteroscopy prior to the first IVF is not recommended, as well as after repetitive implantation failures.

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Case Report

GASTROSCHISIS ASSOCIATED WITH COLONIC ATRESIA - CASE REPORT

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Abstract. *We present an extremely rare case of gastroschisis associated with colonic atresia, anomaly seen in only 1.5-5% of all gastroschisis patients. Surgery for this anomaly can be very difficult due to the concomitant inflammation or other anomalies (mesenterium communae, volvulus, etc). Although prolonged intestinal dismotility is expected in these patients, mechanical obstruction must not be excluded in postoperative course, as it is explained in this case. Our case report demonstrates that primary colonic anastomosis is safe and well tolerated surgical procedure for these patients and that obstructive ileus is possible days after primary surgery and must not be overlooked.*

Key words: *gastroschisis, colonic atresia, treatment.*

Introduction

Gastroschisis (laparoshisis) is a rare congenital defect, which is characterized by abdominal organ prolapse through the small defect of the abdominal wall, usually localized to the right from the umbilicus. Untill today the exact cause and pathogenesis of gastroschisis remain unclear. Prolapsed organs can be small intestine, colon, liver, spleen, stomach, sometimes the ovaries. In most cases gasroschisis is an isolated defect [1] About 5-22% of infants born with gastroschisis will have associated anomalies of the digestive tract, such as atresia or stenosis of the small intestines or colon, malrotation, etc. [2].

Case report

A 37-week-old term male newborn, with prenatally diagnosed gastroschisis was born via uncomplicated vaginal delivery, with body weight of 3150 grams and APGAR scores 9/10. Abdominal wall defect to the right from umbilicus was detected with narrow aperture od 2 cm in diameter and with prolapsed abdominal organs (mostly small and large bowel) (Figure 1).

After initial oropharyngeal aspiration and reanimation, all prolapsed intestines were carefully wrapped in sterile warmed gauze, to prevent iatrogenic volvulus, and the patient was transfered to our neonatal intensive care unit (NICU) for further treatment. After standard preopeative preparation, blood tests, abdominal ultrasound and abdomen and chest radiography, the patient was considered an appropriate candidate for reduction of gastroschisis and primary closure of abdominal wall. Nasogastric (NG) tube was placed for bowel decompression, and there was no meconium after gentle rectal irrigation. Furthermore, by "milking" of dilated bowel loops, the obstruction caused by transverse colon atresia was noticed (Figure 2), and solved by two-layers end-oblique anastomosis of the colon, establishing the intestinal continuity (Figure 3).

The remaining prolapsed abdominal organs were reduced into the abdominal cavity without increasing intra-abdominal pressure. The abdominal defect was primarily closed without complications and the patient was transferred to the NICU. Antibiotic therapy and total parenteral nutrition (TPN) were administered. Postoperatively, daily rectal irrigation started on day 7 in order to activate bowel motility, but without signs of spontaneous meconium evacuation. NG tube even 14 days after surgery evacuated abundant gastric residuum. Ultrasound exami-

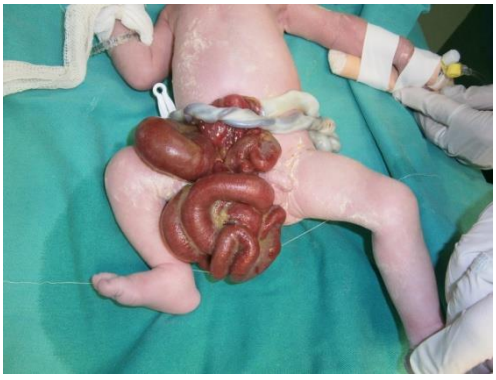

Fig. 1 Patient with gastroschisis. Eviscerated organs are mainly small bowel and colon.

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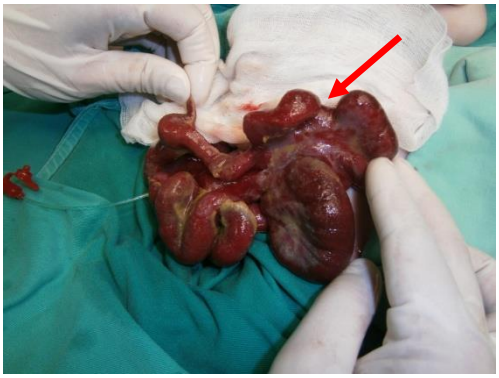

Fig. 2 Atretic colon at in the level of colon transversum (arrows), about 30 cm from appendix vermiformis (between surgeon's fingers)

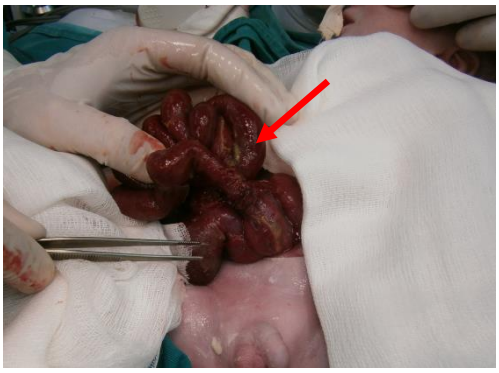

Fig. 3 Primary end-oblique colonic anastomosis (arrow)

nation described extremely dilated aperistaltic bowel loops with lots of free fluid in the abdominal cavity, and air-fluid levels on X ray. Relaparotomy was done fifteen days after primary surgical repair of gastroschisis, discovering "kinking" of colonic loop just few centimeters proximal to the anastomosis site, blocking intestinal passage. Intestinal passage was reestablished immediately after adhesiolysis and the anastomosis was intact. The patient was again transferred to NICU. Further postoperative course was uneventful and the patient was discharged from hospital after 32 days. He has been regularly monitored since then. The boy is now 4 years old with no complications so far.

Discussion

Gastroschisis is congenital ventral body wall defect with increasing prevalence and incidence of 1 per 2000 live births [3]. Isolated colonic atresia represents a very uncommon entity and the rarest cause of neonatal intestinal obstruction with the incidence of 1 per 20.000 live births and it comprises about 1.8-5% of intestinal atresias. Furthermore, the simultaneous occurrence of these two entities is extremely rare. Diagnosis of atresia is often difficult at the initial exploration of the prolapsed intestines because of inflammatory coating covering the bowel, resulting in overlooked associated intestinal atresia in patients with gastroschisis [4].

The pathogenesis of gastroschisis still remains controversial. Various theories and hypotheses regarding this defect are discussed in the literature [5,6], but vascular disruption theory is the most common. According to this theory, gastroschisis is a result of premature involution of right umbilical vein leading to ischemia and forming the weak spot (mesenchymal defect) at the junction of body stalk and future abdominal wall [7]

Considering the extremely rare association of two entities (gastroschisis and colonic atresia) there are no larger series to describe precisely the pathogenesis. However, vascular insult or strangulation of eviscerated intestines in pinched abdominal ring with intestinal infarction strongly support the vascular hypotheses. Intestinal atresia is a consequence of ischemic injury caused by extrinsic mesenteric vascular obstruction in narrow abdominal ring or in utero volvulus [8].

Although intestinal atresias can be diagnosed synchronously with gastroschisis on antenatal ultrasound, in most cases atresias are diagnosed postnatally, even after primary abdominal wall closure, due to the early feeding intolerance during recovery. Contrast radiographic studies can mark the atretic portions of intestines.

Generally, the most important consideration for the surgeon is to find the most adequate surgical approach for treating this small group of patients. Restoring normal bowel function remains the focus of care, and largely depends on the assessment of reactive alterations of the intestine. Intestinal atresia significantly increases morbidity and mortality, so surgeons are still debating about the best method for treating infants with gastroschisis and concomitant atresias. There is no perfect and unique method, and treatment of colonic atresia can be quite challenging. Kronfly published studies suggesting that bowel resection with primary anastomosis is a reasonable treatment for infants with gastroschisis and associated colonic atresia proximal to the splenic flexure, and a colostomy with delayed anastomosis for colonic atresia distal to the splenic flexure [9].

Naturally, surgeons should not insist on primary anastomosis and rational surgical assessment is essential. Intestinal repair at the first operation is sometimes possible and depends on the severity of the inflammatory coating. Unfortunately, this scenario is rarely feasible. If patients are unable to undergo primary anastomosis, restoration of bowel function must be delayed. In this circumstance, the combination of primary abdominal wall closure and enterostomy is the most appropriate strategy. Stoma closure should be planned for second stage, after few weeks, depending on the child's condition. When patients with such anomalies are managed appropriately, outcomes are generally favorable.

In our case we delayed the second operation because we felt that prolonged ileus was the result of gastroschisis itself, which was obviously an incorrect assumption. It is well described in literature that incidence of adhesive ileus and mechanical obstruction is very high, up to 30% [10–12], so delayed intestinal dysmotility should not be understood as a usual complica-

tion in the postoperative course, but should raise suspicion on possible mechanical obstruction.

Conclusion

The management of gastroschisis associated with intestinal atresias has improved in recent decades mainly due to improved care in NICU, TPN and plenty of different individualized surgical approaches available, regarding the intestinal status. Although prolonged intestinal dysmotility is expected in these patients, mechanical obstruction must not be excluded in postoperative course.

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Case Report

SURGICAL TREATMENT OF MENINGIOMA WITH VENTRAL AND VENTROLATERAL LOCALIZATION IN THE REGION OF THE FORAMEN MAGNUM

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Abstract. *Meningiomas localized in the ventral part of the foramen magnum always represent a surgical challenge. Analysis was performed on the surgical approach to meningiomas with ventral localization in the craniocervical region in 6 patients. Two posterolateral surgical approaches were used, depending on whether the tumor was at the level of the foramen magnum or it transited into the cervical spinal canal. In the case of a tumor at the level of the foramen magnum, posterolateral approach was used, with the suboccipital bone removal, and removal of part of the occipital condyles, with the resection of the atlas arch and mobilization of a.vertbralis. In tumors propagated in the spinal canal, the same resection of the occipital bone and occipital condyle was done, with the removal of the atlas and part of the atlantoaxial joint. Due to destabilization, occipitocervical fixation was performed in the second posterolateral approach. The posterolateral approach with the suboccipital removal of the bones and the atlas or, if necessary, with the resection of the occipital condyle or atlantoaxial joint, enables a good ventral separation of the tumor attachment and subsequent gradual complete removal. Fixation is required in the event of a removal of the atlantoaxial joint or removal of more than half of the occipital condyle. Posterolateral approach is an absolute indication in all cases of the ventral and ventrolateral localization of meningiomas in the area of the cervico-occipital junction, because it provides complete visualization of the tumor and allows for its safe removal.*

Key words: *posterolateral approach, occipitocervical junction, meningiomas of the foramen magnum.*

Introduction

Meningiomas in the area of cervico-occipital junction are not a common pathology. Most often, they are localized in the ventral sections of the foramen magnum. The clinical picture that leads to their discovery is directly related to the compression of the brainstem and the lower group of the cranial nerves. The complex anatomical structure of the cervico-occipital junction is an aggravating factor in the planning of a surgical procedure. Therefore, good surgical planning is a prerequisite for a good post-operative outcome. Preservation of nervous and vascular structures is required during the complete removal of meningiomas, due to their benign nature. By using the posterolateral approach to the ventral part of the craniocervical junction, good visualization of neurovascular structures and tumor mass is enabled [1–3].

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Material and Methods

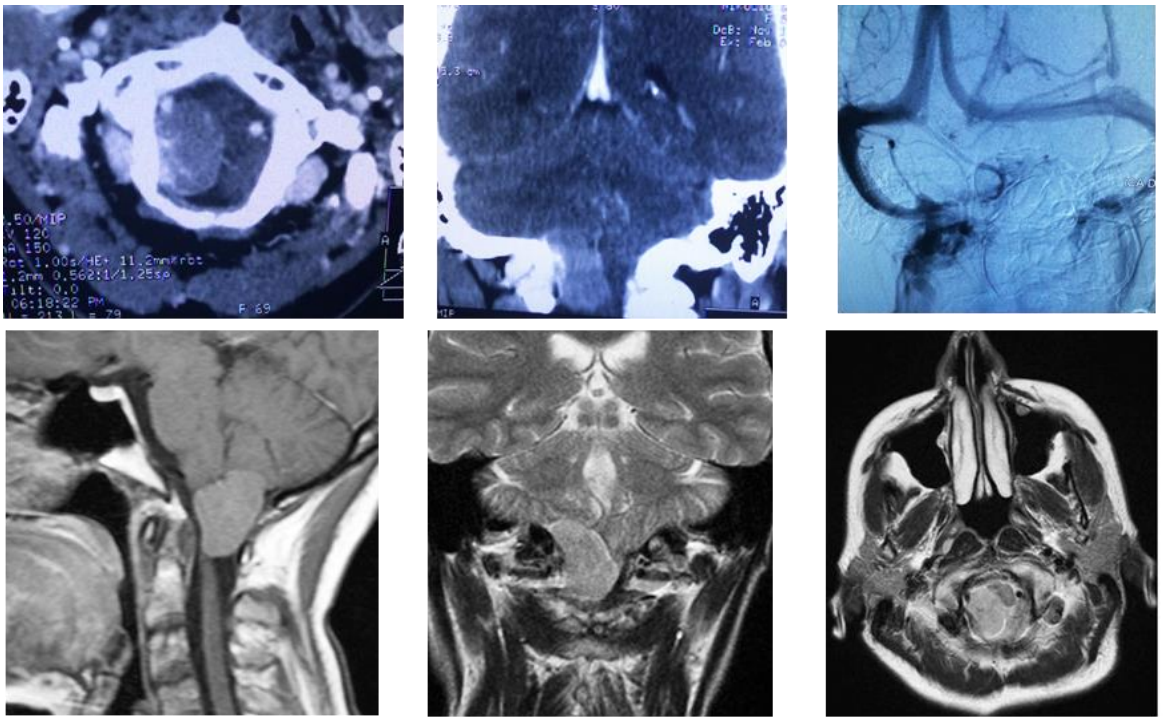
A posterolateral surgical approach was analyzed in 6 patients who were operated on for the meningioma in the foramen magnum region. Surgery was performed on 4 women and 2 men. The clinical picture ranged from difficulty swallowing, difficulty walking, headache, buzzing in the ears, vomiting; it should be noted that 6 to 24 months elapsed from the onset of symptoms to diagnosis. The diagnosis included MSCT of the cranium, brain MRI, and PAN-angiography. The relationship of the tumor with the brainstem, nervous elements and vascular elements was analyzed preoperatively.

Two posterolateral approaches were used:

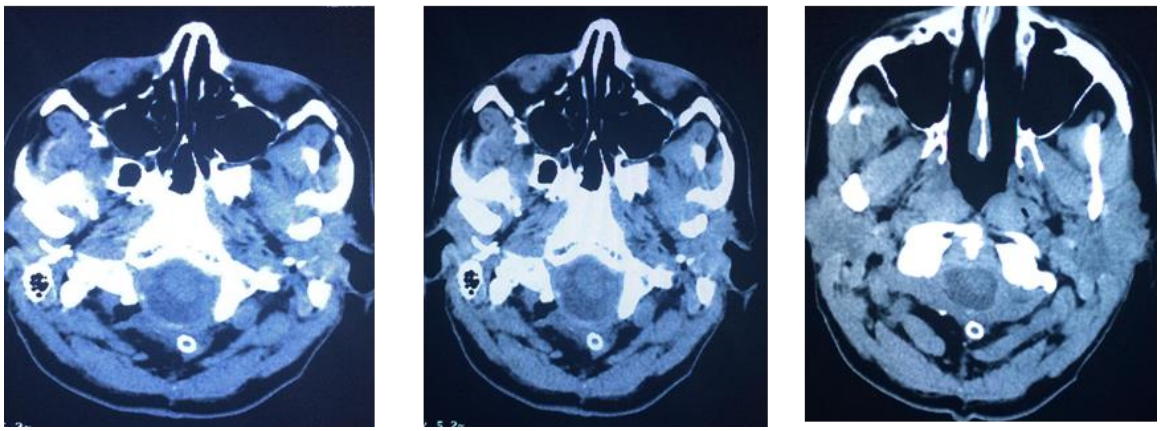
The first posterolateral approach accessed the tumors which were localized in the foramen magnum region, but did not extend below the level of the first cervical vertebra. The patient was placed in a sitting position with a slight flexion of the neck forwards and rotation of the head to the side from which the tumor process was accessed. After removing the muscle by a cut in the form of the reverse letter L, the atlanto-occipital membrane was accessed and the atlas arch was completely dissected up to its juncture with the atlanto-occipital articulation. A.vertebralis was completely mobilized. Part of the occipital bone above the foramen mag-

num on the side of the surgical access was removed; if necessary, part of the occipital condyle was also removed together with a part of the mastoid, and releasing of the sagittal sinus was done. The incision of the dura was done in the area of the atlanto-occipital membrane with an upward and downward expansion. After opening the dura, all neurovascular elements were completely visualized. The technique of precise microscopic separation of n. accesorius and n. hypoglossus from the tumor, with the mobilization of a.vertbralis [4] allows good visualization of the ventral part of the tumor and its attachment. For accessing the tumor, the windows between the accessory branches and the lower group of nerves are used. The tumor is always first separated from the attachment, preferably as a whole, and then removed piecemeal. It is essential that no traction of the nerves is made, although it is allowed to move them. There is always an arachnoid block [5] between the tumor and the brainstem, which enables secure preparation of the tumor and its separation from the brainstem (Fig. 1).

The second posterolateral approach was used to tumors that ran below the level of the first cervical vertebra. The approach is identical to the first case, but the expansion of decompression of the bones is performed to a greater or lesser extent, if necessary, with unilateral removal of the atlanto-axial (C1-C2) articulation. The principle of tumor removal is identical. Because of the destabilization of the articulations, the cervico-occipital stabilization is always performed (Fig. 2).

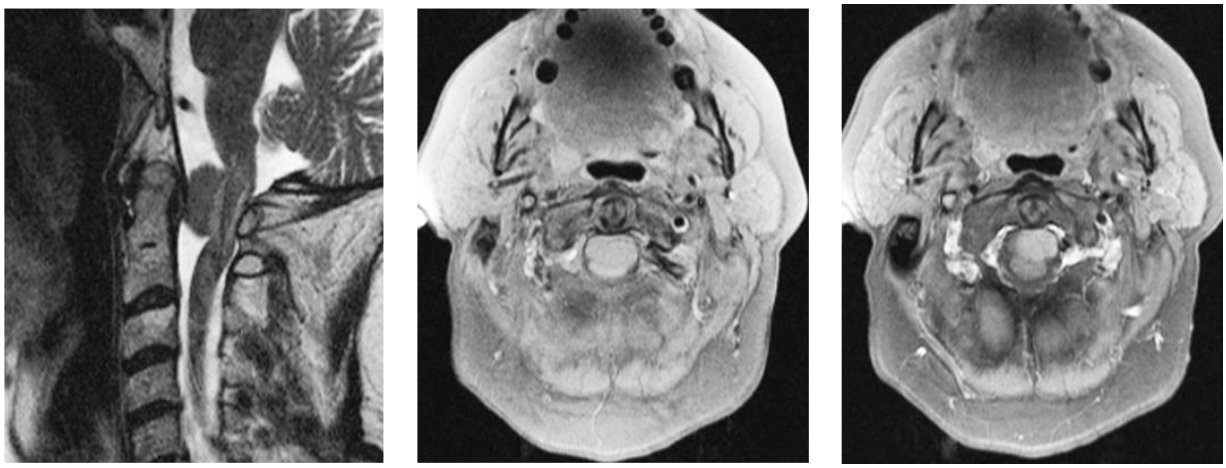


Preopertive MSCT, angiograph and NMR

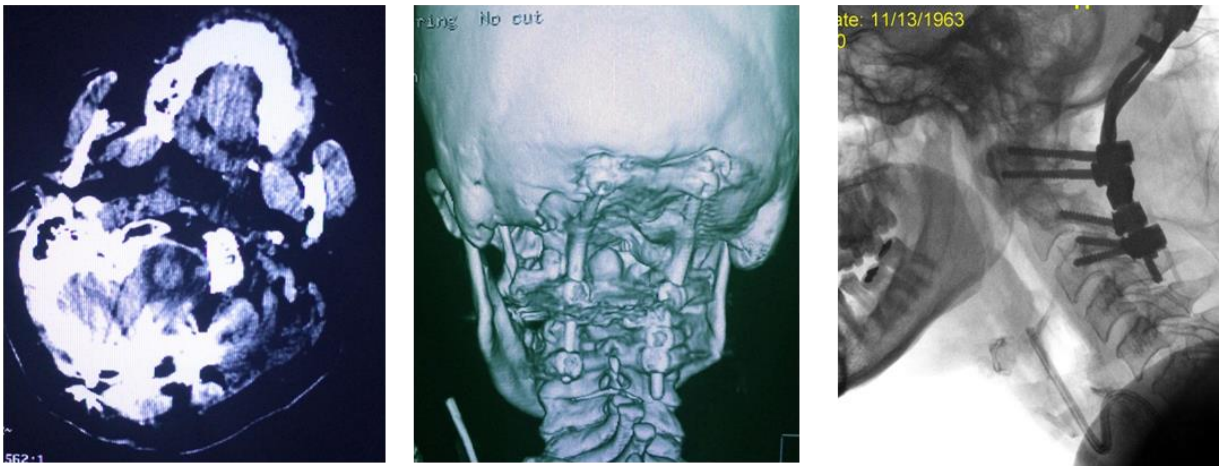


Postoperative MSCT / complete tumor removed

Fig. 1 Posterolateral approach with the complete removal of the lateral part of the atlas with the release of a. vertebralis and atlantooccipital joint



Preoperative NMR



Postoperative MSCT/ complete tumor removed/ removing atalntoxial joint/ occipitocervical stabilization

Fig. 2 Posterolateral approach with the complete removal of atlas with the release of a.vertbralis, partial removal of atlantoaxial joint and cervicooccpital stabilization

Results and Discussion

After the resection of the bone and ligament structures, the posterolateral approach allows for good visualization of the tumor, its attachment and relationship with the neurovascular elements. Such an approach enabled complete removal of the tumor without damaging the surrounding structures, which is why the postoperative course was without neurological complications in all patients [6,7].

For smaller tumors, the posterolateral approach, with the resection of the atlas arch and mobilization of a. vertebralis, allows good visualization of the ventral part of the occipito-cervical junction and complete removal of the tumor [8,9].

For larger tumor processes, the posterolateral approach must be expanded by a variable degree of resection of the occipital condyle and atlantoaxial articulation. This approach enables complete visualization of all neurovascular elements with the brainstem, but requires that cervico-occipital stabilization be performed upon ending the tumor surgery, due to destabilization in the articular systems. [10–12].

In 4 patients, the posterolateral approach was used with the atlas resection, which did not require stabilization. In all patients, the tumor was completely removed. One patient had a short-term problem with swallowing, but these symptoms disappearedafter a month. The other patients were without postoperative neurological problems. Residual neurological problems of the hemiparesis and muscular weakness type retreated over a period of several weeks to three months after surgery. There were no postoperative problems with moving the head in all directions.

In 2 patients with cervico-occipital fixation, limited neck mobility was present, but this did not significantly affect their daily life activities [13–15].

There were no neurological postoperative complications. It should be noted that bleeding was minimal during surgery, despite the fact that in some cases tumor vascularization was significant.

Conclusion

The basic surgical principle is to reach a surgical substrate with minimal damage to the healthy structures and, most importantly, remove it without damaging the vital neurovascular structures. Within each surgical approach, the removal of the bones and ligament structures must be performed with respect to the size of the tumor being operated.

Surgical approaches for such tumor localization, whose implementation will increase the chance of major bleeding, infection, incomplete recovery, and the need for later reconstruction of the surgical entry, must be kept to a minimum or even eliminated [16–18].

The posterolateral approach to the ventral processes in the foramen magnum region absolutely allows good visualization of the ventral part of the cervico-occipital junction, good visualization of the relationship of the tumor with the neurovascular elements, and the complete removal of the tumor process [19–21].

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