Vol. 9
N°3
November 2008.
ISSN 1820 – 8665

Serbian Journal of Experimental and Clinical Research

2000

Serbian Journal of Experimental and Clinical Research

2008

Print
Medical Faculty, Kragujevac

Indexed in
EMBASE/Excerpta Medica, Index Copernicus, BioMedWorld, KoBSON, SCIndeks

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SJECR is a member of WAME and COPE. SJECR is published at least twice yearly, circulation 250 issues The Journal is financially supported by Ministry of Science and Technological Development, Republic of Serbia
ISSN 1820 – 8665

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UDK 616.13-004.6-02 / Ser J Exp Clin Res 2008; 9 (3): 81 – 88

CARDIOVASCULAR ADIPOBIOLOGY: A NOVEL

HEART-ASSOCIATED ADIPOSE TISSUE IN CARDIOVASCULAR DISEASE

ABSTRACT

This article provides a conceptual framework for the possible role of (i) periadventitial adipose tissue, and (ii) epicardial adipose tissue in the pathogenesis of cardiovascular disease. Traditional concepts of atherogenesis are focused on luminal surface, where "inside-out" inflammatory events trigger the extravasation of immune cells and the accumulation of lipids, smooth muscle cells and matrix components leading to atherosclerotic plaque formation. However, increasing evidence supports a new concept of an "outside-in" responses, involving periadventitial adipose tissue, herein referred to as tunica adiposa, and epicardial adipose tissue, these two adipose loci functioning as secretory tissues. Thus, a paracrine signals originated from these tissues could be transmitted into both the coronary artery intima and the myocardium. The present review highlights the possibility for tunica adiposa and epicardial adipose tissue to play an important role in an "outside-in" signaling in the development of atherosclerosis and cardiomyopathy. In effect, adipose-targeted pharmacology and non-invasive measures might provide novel clinical insights into cardiovascular adipobiology. Data of adipose-derived adipokines, including neurotrophic factors and neuropeptides, are also presented, raising a hypothesis of neuroendocrine potential of adipose tissue; it may also be instrumental in the pathogenesis of cardiovascular disease.

Key words: adipokines, atherosclerosis, epicarial adipose tissue, neurotrophic factors, neuropeptides, periadventitial adipose tissue

INTRODUCTION

"Ask yourself for each of your thoughts: is it a new one?"
Carl Gustav Jung (1875-1961)

Contemporary human lifestyle related to feeding and physical activity can lead to an increasing accumulation of adipose tissue. Hence the incidence of obesity and related cardiovascular diseases including atherosclerosis, hypertension and metabolic syndrome is increasing dramatically in all countries of the world. The overwhelming influence of these diseases contributes to a decreased quality of life as well as significant economic consequences. Hopefully, there would be great benefits if research could achieve effective prevention and therapy for cardiovascular diseases.

Work over the past several years has revealed that adipose tissue plays a pivotal role in controlling whole-body lipid and glucose homeostasis in both normal and disease state, visceral adipose tissue accumulation and inflammation-related insulin resistance being considered as the common denominators of the development of cardiovascular disease (1-4).

Atherosclerosis is a disease affecting mainly "large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain, or extremities, resulting in infarction" (5). In atherogenesis, the response-to-injury hypothesis of Russell Ross proposes that lymphocyte and monocyte extravasation into the intima, and vascular smooth muscle cell proliferation (5) and oversecretion of matrix molecules (6) are the key events in the initiation and development of atherosclerotic plaques. Because advanced intimal lesions lead to luminal loss, resulting in infarction, the intima is considered by many authors the most important vascular area involved in atherogenesis. Recently, growing evidence, however, rises the possibility of adventitial (7-10), peri-

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adventitial adipose tissue (PAAT) (11-14) and epicardial adipose tissue (EAT) (15-19) pathways in the cardiovascular injury response.

Here we emphasize that heart-associated adipose tissue represented by PAAT (herein referred to as *tunica adipose*) and EAT are an additional example of the secretory potential of the heart, the first one being atrial natriuretic peptide secretion initially suggested by 1964 George Palade's electron microscopic description of "specific granules in atrial mucles" (20). Further, there is at present evidence that the sharing of ligands (growth factors, cytokines, and adipokines) and their receptors constitute a molecular language of neural, immune and adipose cells (21-29). Note that perinodal adipose tissue is a newly recognized feature of the lymph node (30), thus making lymph nodes an excellent example of adipo-immune paracrine interactions. Other examples include orbital adipose tissue in Graves' thyroid-associated ophthalmopathy (31), mammary gland-associated adipose tissue in breast cancer (32), and artery- and heart-associated adipose tissue in cardiovascular diseases (33-42). Briefly, PAAT and EAT could represent another examples of adipoparacrinology (43). Table 1 presents heart-associated adipose tissue loci.

Table1. Heart-associated adipose tissue loci*

Epicardium/subepicardium
Pericardium
Atrial septum
Coronary arteries (proximal segments)

* Heart adiposity should be distinguished from obesity-related lipotoxic cardiomyopathy, in which excessive fat accumulates inside cardiomyocytes (19).

SECERTORY NATURE OF HEART-ASSOCIATED ADIPOSE TISSUE

The white adipocyte has long been recognized as the main producer of triglycerides during feeding, which are stored in highly concentrated form in a single large-sized lipid droplet. Whereas during fasting, the triglycerides are hydrolyzed and released into the blood circulation as free fatty acids (and glycerol), which are transported to other tissue to be oxidized in mitochondria as an energy source. Note that circulating fatty acids mediate insulin resistance (in skeletal muscle) in obesity and related diseases (1). By contrast, a high content of mitochondria (which produces the brown color) and of the uncoupling protein-1 allows the brown adipocyte to generate heat, thus being specialized primarily for the regulation of non-shivering thermogensis.

Arguably, the most momentous changes that have occurred in the field of adipose tissue study have been the discovery and elucidation of its endocrine and paracrine function. Initial insights into this new biology of adipose tissue resulted from the discovery of leptin in 1994. The secretory potential of adipose tissue is executed by adipocytes as well as non-adipocyte cell types such as tissue matrix cells, stromovascular cells and associated macrophages, mast cell and lymphocytes (43-51). All these cells secrete a large number of proteins designated adipokines (Table 2). Adipokines play important roles in the pathogenesis of a various diseases beyond obesity. Accordingly, adipobiology of disease has emerged as a novel field of studies, which has enjoyed explosive growth in the past 10 years in basic, translational and clinical medicine (11-13,43-56). We argue that each intracellular step of adiposecretion, including synthesis, sorting, storage, and exocytosis, as well as adipokine receptors, may provide novel targets in adipopharmacology of cardiovascular disease (25-27,45,49).

Table 2. A selected list of adipose tissue-derived mediators, as related to cardiovascular disease

Pro-inflammatory adipokines
C-reactive protein, serum amyloid A, haptoglobin, leptin, IL-1, IL-6, IL-18, MCP-1/CCL2, IL-8/CXCL8, tumor necrosis factor-α, plasminogen activator inhibitor-1, IGF1, angiotensin II
Anti-inflammatory and metabotrophic adipokines
Adiponectin*, IL-1 receptor antagonist*, IL-10*, leukemia inhibitory factor (LIF)*, metallothionein 1-3, ciliary neurotrophic factor, NGF*, BDNF*, transforming growth factor-β*, cardiac natriuretic peptide, adrenomedullin*, angiopoietin-like protein 4*
Vasodilators
Adipocyte-derived relaxing factor, nitric oxide (NO)*, adiponectin*, cardiac natriuretic peptide, adrenomedullin*
Vasoconstrictors
Superoxide anion, angiotensin II, endothelin-1
Other mediators
Complement-C1q TNF-related protein-1 (CTRP1), kisspeptide, estrogens*, free fatty acids, prostaglandins

* At present, antiatherogenic and/or metabotrophic effects of these mediators are reported (114 for adrenomedullin; 115 for LIF; 116 for angiopoietin-like protein 4; references for other mediators are indicated in the text). For stimulation of aldosterone production by CTRP1 (117), for receptor-mediated actions of free fatty acids (118); for metallothioneins (51,119-121); for angiopoietin-1 (122); for NGF/VEGF (123,124); for kisspeptide-1 (125); for NGF, BDNF and other metabotrophic factors (103,112,126)

A long standing paradigm holds that the vascular wall consists of three concentric tissue coats (*tunicae*): intima, media, and adventitia. Almost every systemic blood vessel, including arterioles (13), are surrounded by PAAT, which had been mainly considered as a mechanical support for vascular wall. Note that PAAT and EAT, also pericardial adipose tissue (57-59) and atrial septum-associate adipose tissue (60), are a visceral adipose depot, which have not been studied thoroughly as visceral abdominal adipose tissue and subcutaneous

adipose tissue. However, PAAT and EAT could influence coronary and myocardial biology because there is no fibrous layer to impede diffusion of bioactive molecules such as adipokines between these adipose tissues and the underlying coronary artery as well as the myocardium. We for the first time conceptualized a "possible interactive involvement of intima, adventitia and associated adipose tissue" in the pathogenesis of atherosclerosis (11,12). Indeed, an extensive body of work has revealed that adipose tissue (mainly its white phenotype) expresses not only metabolic, but also endo-, auto-, intra- and paracrine ("vasocrine") phenotypes (Fig. 1), particularly in adipobiology of disease (45,52-56). This new biology is achieved through synthesis, storage, and release (that is, Palade's paradigm of cell protein secretion) of adipokines (44-47,49,50), which include more than hundred highly active signaling proteins abundantly secreted by inflamed adipose tissue. Adipokines may contribute to alterations of vascular functions, including low-grade inflammation (2,3,17-19,48), vascular tone (52,53,55), growth of smooth muscle cells (55) and insulin resistance (13,22,44). Furthermore, mast cells (12,21,27), lymphocytes and dendritic cells (22,30) and macrophages (19,23,24,27) populate adipose tissue, including PAAT and EAT (16,17,19,61-63) (Fig. 2).

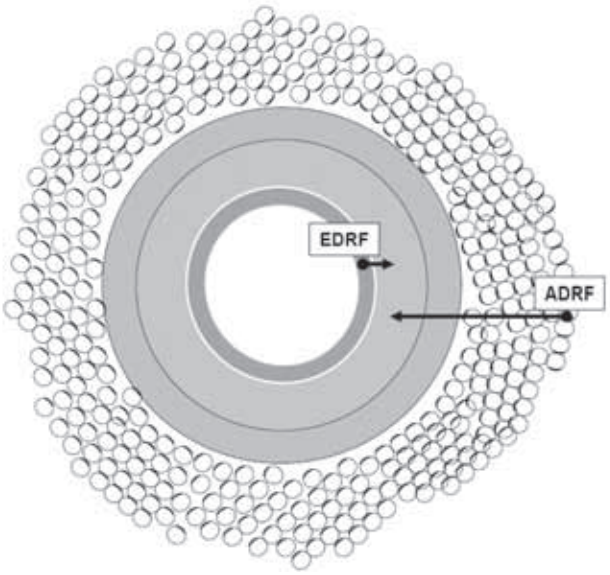

Figure 1. Schematic presentation of vascular wall composed of four tissue coats (*tunicae*): intima, media, adventitia, and adiposa. Arrows show that *tunica media* is a target for at least two vasorelaxing factors, endothelium-derived relaxing factor (EDRF) and adipocyte-derived relaxing factor (ADRF).

Taken together, we proposed that PAAT could, at both functional and structural levels, be considered *tunica adiposa*; that is, the fourth, outermost vascular coat (36). By analogy with endothelial dysfunction, the term adipose dysfunction is consequently introduced in vascular and metabolic biology (36,37,44).

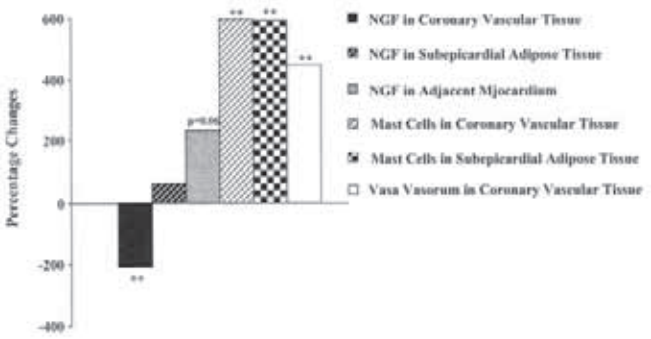

Figure 2. Changes in amount (pg/g) of nerve growth factor (NGF) and in number of mast cells in selected human cardiac tissues expressed as percentage of controls. From 63.

One aspect of the role of *tunica adiposa* is whether it facilitates or inhibits the process of atherogenesis. From human coronary atherosclerosis we know that the proximal coronary segments, particularly the left anterior descending (LAD) branch, are surrounded by subEAT and are atherosclerosis-prone as compared to the distal, intramyocardial, adipose coat-free, atherosclerosis-resistant coronaries (33,61). Reminding Carl Jung's concept of cryptomnesia expressed in the motto of present article, it must be mentioned that in 1933, Smith and Willius (cited by 19,59) reported their results obtained from autopsies on 136 obese subjects and suggested a functional relationship between the EAT and the LAD coronary artery. The authors found that "in most instances, a definite relationship between the excess of epicardial fat and the degree of general obesity occurred." Another important reason for subEAT to serve as an example of (a large-sized) *tunica adiposa* is the close association of the coronary vasculogenesis with epicardium-derived mesenchymal cells. These cells invade the subepicardial matrix and differentiate into coronary vascular smooth muscle and endothelial cells (64). Last not least, segments of coronaries lacking PAAT and thus being bridged by myocardial fibers are protected against the development of atherosclerosis (see 19,33). However, when EAT and probably PAAT are totally absent in congenital generalized lipodystrophy, coronary atherosclerosis can still occur. Thus, we should remind ourselves that "a little fat is good" (cited by 12) as well as "the fatter, the better" (65).

Further, in 1962 Schwartz (cited by 7) wrote with respect to the presence of adventitial mononuclear cell infiltration of atherosclerotic vessels: »It is perhaps surprising that such prominent cellular accumulation should have received so little attention... Nevertheless, since cellular infiltration of the adventitia shows such a constant relationship to the presence and degree of plaque formation, it should not be disregarded«. Today, this statement may also address PAAT (*tunica adiposa*). Thus, PAAT may indeed represent one of many paths leading to atherosclerosis (66).

NEUROTROPHINS AS ADIPOKINES

Within the vascular wall, smooth muscle cells comprise the primary target of the sympathetic neurons and, respectively, serve as the main source of nerve growth factor (NGF) (61-63 and references therein). Recent evidence shows that plasma and tissue levels of the neurotrophins NGF and/or brain-derived neurotrophic factor (BDNF), which is also produced by adipose tissue (67), are decreased in various cardiovascular diseases (61-63,68-71, cf. 72,73). Noteworthy, also decreased are the plasma levels of adiponectin (17,74,75), an "old" antiatherogenic (48) and a novel vasorelaxing (53) adipokine.

Given the key role of inflammation and fibrosis in the development of atherosclerotic lesions, what role, for example, might *tunica adiposa* and EAT play in the process of atherogenesis? The expansion of adipose tissue seen in obesity is associated with an imbalanced secretion represented by an enhanced release of inflammatory adipokines and by decreased release of antiinflammatory adipokines (Table 2). Such an "enemy-or-friend" (36), "bystander or player" (38,39) or "double role" (40,41) secretory capacity of PAAT requires specific pharmacological manipulation, which aims at boosting the production and/or receptor sensitivity of anti-inflammatory/metabotrophic adipokines. Further, both leptin (76,77) and NGF (78-81) accelerate skin wound healing and thus raise a pressing question of whether this may also be the case with atherosclerotic vascular wound (see 5 to remind Russell Ross's paradigm of atherogenesis). Note that there is an increased tissue levels of NGF in diabetic skin wound (79,80) as well as ischemic cardiac tissue (82), whereas exogenous administration of NGF improves the healing process in both tissues. Likewise, treatment with NGF (83) or adiponectin (84) resulted in a significant upregulation of IL-10, an antiinflammatory cytokine. These data may also be relevant to a potential therapeutic effect of both NGF and adiponectin in atherosclerosis, an inflammatory, IL-10-deficient disease (5).

Clearly, the importance of local and systemic secretory involvement of both *tunica adipose* and EAT requires further evaluation in cardiovascular pathology. Understanding the paracrine signaling issued from PAAT and EAT might eventually lead to therapeutic and preventive strategies that will improve human vascular and metabolic health (85,86).

NEUROPEPTIDES AS ADIPOKINES

As neurotrophins, a large number of neuropeptides are also produced by adipose cells and exert extraneuronal effects, including on glucose, lipid and energy metabolism. Examples include substance P (87), neuropeptide tyrosine (NPY) (88-90) and other neuropeptides (91-96). In the same vein, most hypothalamic and pituitary neuropeptides, hormones and releasing factors, termed "adipotrophins" (97), express their receptors in adipose tissue, creating hypothalamic-pituitary-adipose axis (98-100). Further, (i) amino acid neurotransmitters such as glutamate and gamma-aminobutyric acid and their receptors are expressed in adipose tissue (101), and (ii) multipotential stem cells are associated with perivascular adipose tissue (102).

Taken together, the adipose-expression of neuropeptides/receptors and neurotrophic factors such as NGF (103-105), BDNF (67,105) and ciliary neurotrophic factor (103 and references therein) raises a possibility of adipose tissuežs neuroendocrine potential, which may also be implicated in the pathogenesis of cardiovascular disease.

Nevertheless, one thing stays much clear: in basic cardiovascular research, we should no longer cut neither adventitia, nor *tunica adiposa* and EAT, but keep them attached and in place, and subject to thorough examination. "Non-touch harvesting technique" in coronary artery bypass surgery (106) is a clinical example of such an adipoprotective approach (see 107-110). Further, non-invasive measures at present are focused on vascular functions and structures such as flow-mediated vasodilation and intima-media thickness, whereas measurements of the thickness of adventitia and adiposa are neglected; indeed, measurements of EAT have been recently performed in clinical practice (15,111). In future, echographic, MRI and other non-invasive assessments of these cardiovascular coats may form a rational for identifying high-risk population susceptible to atherosclerosis, and monitor vascular wall changes during follow-up studies and therapeutic trials.

POST SCRIPTUM

In humans, white adipose tissue is partitioned into a few large depots, including visceral and subcutaneous location, and many small depots associated with heart, blood vessels, lymph nodes, ovaries, eyes, kidneys, adrenal glands, also located in liver, skeletal muscles and mammary glands (30-33,45). Accordingly, *Homo obesus* (112) is currently viewed as a disorder triggering the pathogenesis of a variety of cardiometabolic, liver, ovary, lung, and neurodegenerative (e.g. Alzheimer's) diseases. Adipotopography (fat mapping) is an emerging subfield of adipobiology dealing with localization and amount of adipose tissue in the human's body (113). Thus people may express TOFI (Thin Outside, Fat Inside), TOTI or other phenotypes (Table 3).

TOFI was described by Dr Jimmy Bell, head of the Molecular Imaging Group at Hammersmith Hospital, London, UK. It can be visualized by using current imaging technologies such as echography, computed tomography, magnetic resonance imaging, and proton magnetic resonance spectroscopy. A predictive message of adipotopography is that "being thin does not automatically mean you are

not fat", quoting Dr Bell. The concept of TOFI holds that small adipose depots, when enlarged and activated (by inflammatory, overnutritional or other stimuli), may exert disease-promoting actions over adipose tissue-associated organ(s). Thus, the traditional diagnostic significance of BMI, as well as other anthropometric criteria (waist and hip circumference alike), should be re-evaluated in obesity and related diseases. Importantly, dieting is enough to keep one being thin outside, whereas physical activity prevents the accumulation of internal fat, thus one can be thin inside. In conclusion, TOFI is a Trojan Horse inside the human's body, a pathological phenomenon, whereas TOTI is a healthy adipose phenotype. Briefly, slim or obese, get your fat map.

Table 3. Adipotopography (fat mapping): variations+

TOFI**	thin outside, fat inside
TOTI***	thin outside, thin inside
FOFI*	fat outside, fat inside
FOTI**	fat outside, thin inside

\+ The number of asterisks indicates the quality of cardiometabolic health, as related to adipose tissue**.** Hence, stay TOTI. From 113.

ACKNOWLEDGEMENTS

Work reported in most of our herein cited papers was supported by grants of Dr Luigi Aloe (Institute of Neurobiology and Molecular Medicine, National Research Council, Rome, Italy). The author greatly appreciate the brain-and-heart collaboration of all the coauthors of these papers.

George N. Chaldakov

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UDK 616.832-004-085 / Ser J Exp Clin Res 2008; 9 (3): 89 – 92

REDUCING THE PHAGOCYTIC ABILITY OF MONOCYTES IN PATIENTS WITH MULTIPLE SCLEROSIS

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SMANJENJE FAGOCITNE SPOSOBNOSTI MONOCITA KOD PACIJENATA OBOLELIH OD MULTIPLE SKLEROZE

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Received / Primljen: 29. 05. 2008. Accepted / Prihvaćen: 15.10.2008.

ABSTRACT

Multiple sclerosis (MS) is an immune-mediated disease of the CNS that is characterised by inflammation, demyelination, and axon loss. It is an autoimmune disorder involving inflammatory T cells (CD4+, CD8+) and auto-antibodies against myelin antigens such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). During the process of the autoimmune inflammatory attack in the CNS a large amount of apoptotic T cells is generated. Microglia and blood monocyte-derived macrophages play the most important part in the efficient clearance of these cells. Their ability to engulf the apoptotic cells efficiently is accompanied by an array of anti-inflammatory effects that are important in reaching the remitting phase.

We observed the ability of monocytes to efficiently clear apoptotic T cells in individuals with MS. It was found that the percentage of monocyte-induced phagocytosis of apoptotic lymphocytes as well as the phagocytic potential of monocytes significantly decreased ($p = 0.000$) in people with MS compared to healthy controls. Our results suggest that the reduction in the ability of monocytes to efficiently engulf a large number of apoptotic cells is connected to the inflammatory process in diseases such as MS.

Keywords: phagocytosis, monocytes, T cell, apoptosis, multiple sclerosis

SAŽETAK

Multipla skleroza (MS) je imunski posredovano oboljenje centralnog nervnog sistema (CNS) koju karakteri{e zapaljenje, demijelinizacija i gubitak aksona. To je autoimunsko oboljenje u kome učestvuju zapaljenske T ćelije (CD4+, CD8+) i autoantitela prema antigenima mijelina, kao {to su mijelinski bazni protein (MBP), proteolipidni protein (PLP) i mijelinski oligodendrocitni glikoprotein (MOG). Tokom ovog autoimunskog zapaljenskog procesa stvara se veliki broj apoptotičnih T ćelija u CNS-u. Mikroglija i makrofagi (koji potiču od monocita) imaju najvažniju ulogu u efikasnom uklanjanju ovih ćelija. Njihova sposobnost da fagocituju apoptotične ćelije je praćena nizom antizapaljenskih efekata. što je značajano za ulazak bolesti u fazu remisije.

U ovoj studiji smo pratili sposobnost monocita kod ljudi obolelih od multiple skleroze (MS) da efikasno uklanjaju apoptotične T ćelije. Prona{li smo da je procenat monocitne fagocitoze apoptotičnih limfocita i fagocitni potencijal monocita značajno smanjen ($p = 0,000$) kod pacijenata obolelih od MS-a. Ovakvo smanjenje sposobnosti monocita da efikasno fagocituju veliki broj apoptotičnih ćelija je povezano sa zapaljenskim procesom u oboljenju kao što je MS.

Ključne reči: fagocitoza, monocit, T limfocit, apoptoza, multipla skleroza

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated disease of the CNS that is characterised by inflammation, demyelination, and axon loss. Traditionally, MS is considered to be a $CD4^+$ T helper 1 (Th1)-mediated disease (1, 2), but these T cells alone are not sufficient to produce the typical neuropathology of the disease (3, 4). It has been shown that MS is an autoimmune disease with a heterogeneity of pathogenetic mechanisms responsible for myelin destruction (5, 6). Myelin is damaged due to an autoimmune attack consisting of several pathways and molecules. The most important myelin antigens are myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) (7-9).

In actively demyelinating lesions, T cells (CD4+, CD8+) and macrophages are dominant cells that participate in the inflammatory reaction (10 - 12). Apoptotic cell death of inflammatory T cells is an established mechanism to terminate an autoimmune inflammatory response in the rodent or human CNS. The apoptosis leukocytes lose the ability to release toxic components through receptor signals so that they are confined with-

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in an intracellular protein membrane (13). The efficient clearance of apoptotic cells in the CNS prevents them from going into secondary necrosis (14, 15). When apoptotic cells go into secondary necrosis, they release their own intracellular contents, which releases many harmful substances and causes damage to the surrounding tissue and induces inflammatory disease (16-18). Cationic protein release following secondary cell necrosis further inhibits the phagocyte clearance of apoptotic cells and causes lower interstitial pH that could activate lisosomal enzymes (16). Furthermore, proteinase inhibitors may be inactivated by oxidation in tissues with dying cells (19).

In addition, the efficient clearance of apoptotic cells prevents the presentation of neoantigene determinants that are formed under the influence of caspases. This can trigger autoimmune disease in sensitive individuals.

Microglia and blood monocyte-derived macrophages constitute the primary phagocytic cells in the brain and they are the primary candidate for the clearance of apoptotic T cells (20). It is important to mention that no quantitative data has been published and no clear distinction has been made between macrophages and microglia. We therefore investigated the ability of monocytes to efficiently clear apoptotic T cells in individuals with MS. Our data demonstrate the lower ability of monocytes to engulf apoptotic T cells in patients with MS compared to healthy controls.

MATERIALS AND METHODS

Patients and controls

Twenty patients with MS were recruited at the Department of Neurology of the Medical Faculty at the University of Kragujevac. All patients had been diagnosed to have relapsing-remitting MS according to the revised diagnostic criteria (21). Patients were not given corticosteroids or immunomodulatory therapy for three months prior the blood collection. Seven healthy staff members of the same institution represented the control group. The clinical features of the subjects are summarised in Table 1.

Table 1. Clinical data of patients with multiple sclerosis (MS) and healthy controls

	Controls	Patients with MS
Total number (males)	7 (3)	20 (9)
Age	40.1 ± 9.1	43± 11.8
MS duration (years)	/	11.4± 8.1

Preparation of monocytes

To test their phagocytotic ability, monocytes were obtained from 10 ml of heparinised whole blood of patients with MS and healthy controls. Mononuclear leukocytes were separated by density gradient centrifugation (Histopaque 1077, Sigma, Germany) (14, 22). Cells were washed three times and suspended in Haemacel (Hoechst Marion Rousel, Germany). Mononuclear leukocytes were put in petri dishes with autologous plasma and kept at 37°C in a 5% CO_2 atmosphere for 30 min. After incubation, non-adherent lymphocytes were washed out with Haemacel. Adherent monocytes were slowly removed using a scraper and were then washed with Haemacel. Cell number and viability were determined using AO/EB staining (Sigma, Germany).

Preparation of apoptotic lymphocytes

We used peripheral blood lymphocytes obtained from the patients with chronic lymphocytic leukaemia (CLL) to obtain apoptotic cells. After separation, CLL mononuclear leukocytes were washed three times in RPMI 1640 culture medium (Sigma, Germany) and suspended in RPMI 1640, supplemented with 20% of autologous plasma, and kept at 37°C in a 5% CO_2 atmosphere until further use. Cells were incubated in 100 μg/ml of CHX (Oncogene, Germany) for up to 24 hr at 37°C in 5% CO_2 atmosphere to induce apoptosis. Cell number and viability were determined using AO/EB staining (Sigma, Germany).

In vitro phagocytosis assay

Apoptotic lymphocytes were collected, washed two times in Haemacel to remove CHX, and suspended in 1 ml of Haemacel. To differentiate between tested and apoptotic cells, we induced an increase in the membrane permeability of apoptotic cells by incubating them in a water bath at 80°C for 10 min. As a result, the membranes of early and late apoptotic cells became permeable to EB. Apoptotic cells stained with AO/EB emitted red fluorescence and were later easily distinguished from the green viable cells. After finishing this stage, apoptotic cells were centrifuged and stained with 10 μl of AO/EB. Because of the fast absorption of stains, the excess of stains were immediately removed by washing the cells in 5 ml of Haemacel. The assay was performed by adding $0.1x10^6$ of the tested monocytes and 20 μl of autologous serum. Haemacel was added to make a final volume of 150 μl that was added to $4x10^6$ apoptotic cells. The suspension was then centrifuged for 5 min at 200 g and incubated at 37°C for 1 hr. After the incubation period, the cell suspension was washed once with ice-cold 0.02% EDTA (Merck). Cells were resuspended in 30 μl of cold EDTA and kept at 4°C until analysis.

Fluorescent microscopy analysis

Samples of 10 μl were taken and stained with 0.5μl AO/EB. Apoptotic cells emitted red fluorescence and viable monocytes emitted green fluorescence. Phagocytosed apoptotic cells were visualised as red cells engulfed by tested monocytes with a green nucleus. When using light

microscopy, engulfed cells were seen as circles surrounded by the cell membrane of the monocytes. The percentage of monocytes engulfing at least one apoptotic cell defined the percentage of phagocytosis (PP). The percentage of phagocytosis was determined by the examination of at least 1000 green cells. The absolute number of phagocytes (AN) was calculated by multiplication of PP and the number of monocytes in 1 ml of whole blood. This value was the measurement of phagocytic potential of the tested monocytes.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical significance of the differences between the groups ($p < 0.05$) was evaluated using an unpaired independent samples t-test.

RESULTS

Phagocytosis of apoptotic cells by monocytes

As the previous studies showed, monocytes were capable of engulfing apoptotic cells (23, 24), so a comparative measurement of the percentage of phagocytosis of apoptotic lymphocytes by monocytes and the absolute phagocytic potential of monocytes in the individuals with multiple sclerosis and in the healthy control was conducted in this experiment.

Furthermore, by measuring the percentage of the phagocytosis of apoptotic lymphocytes by monocytes a significant decrease ($p = 0.000$) was found in the patients with MS (6.551 ± 2.665%) in comparison to the healthy control (14.143 ± 3.437 %) (Fig.1). Considering the fact that the better evaluation of the disorder in monocyte-induced phagocytosis is obtained by measuring their absolute phagocytic potential, a comparative analysis of the absolute phagocytic potential of monocytes was conducted. We found that the phagocytic potential of monocytes significantly decreased ($p = 0.000$) in people with MS (0.208 ± 0.083) compared to healthy controls (0.427 ± 0.051) (Fig. 2).

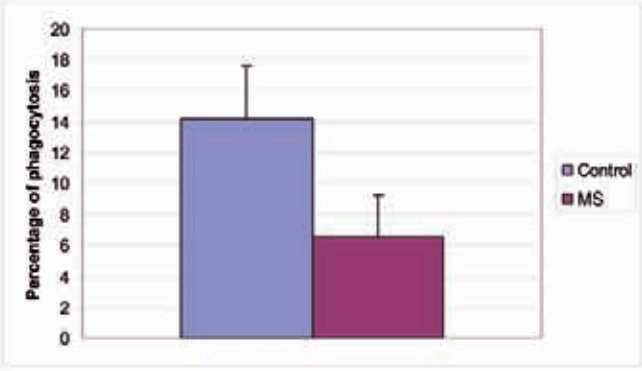

Figure 1. Percentage of phagocytosis of apoptotic lymphocytes by monocytes.

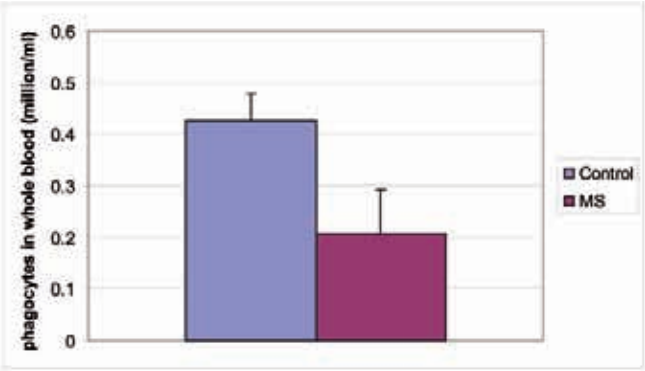

Figure 2. Phagocytic potential of monocytes in 1ml of whole blood

DISCUSSION

A large amount of apoptotic cells is generated during the process of the autoimmune inflammatory attack of the CNS in people with MS. One of the most important mechanisms to the resolution of inflammation in the CNS is the efficient clearance of the large number of apoptotic inflammatory T cells (15) as well as other apoptotic cells such as oligodendrocytes and neurons (25) that are generated during this process. It has been shown that clinical recovery in patients with MS is connected to the loss of inflammatory cells in the CNS and in the peripheral immune system (26, 27).

Brain microglia, along with blood monocyte-derived macrophages, play the most important part in the clearance of apoptotic cells produced in this way (20). The reduction in their ability to efficiently engulf a large number of apoptotic cells in the CNS causes the relapse in MS. We have shown a significant decrease in the phagocytic potential of monocytes in people with MS compared to healthy controls ($p = 0{,}000$). The importance of this process in MS is also documented in the fact that the phagocytosis of apoptotic cells significantly alters the macrophages' states of immune activation, which is accompanied by an array of anti-inflammatory effects.

Many studies have shown that phagocytosis of apoptotic cells by macrophages influences their cytokine secretion. After engulfing the apoptotic cells, macrophages secrete considerably less pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-12, tromboxane B2, leucotriene C4, and granulocyte-monocyte colony- stimulating factor (GM-CSF) (28). This process, however, results in the increased secretion of anti-inflammatory cytokines such as transforming growth factor (TGF)-β1, prostaglandin (PG)E2, and platelet-activating factor (PAF) (28- 30). It is important to note that at the same time, the secretion of IL-10 is decreased. In addition, the release of TGF-β1, PGE2, and PAF affect the macrophages, causing the decrease in pro-inflammatory cytokine secretion (28).

Following the phagocytosis of apoptotic cells, macrophages / microglia reduce the B7-2 co-stimulatory ex-

pression, leading to the inhibition of T cell proliferation (14).

Our findings suggest that the importance of the ability of monocytes to engulf apoptotic cells is fairly significant in disorders such as MS. Future studies investigating the interaction of monocytes and apoptotic cells may provide new important insights into such disorders.

ABBREVATIONS

AO- acridine orange, EB- ethidium bromide, CHX- cycloheximide, CLL- chronic lymphocytic leukaemia, CNS- central nervous system, EDTA- ethylene diaminetetraacetic acid, IL- interleukin, MS- multiple sclerosis

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UDK 616.65-006.6-071 / Ser J Exp Clin Res 2008; 9 (3): 93 – 97

CORRELATION BETWEEN NUMBER OF PROSTATE BIOPSY SPECIMENS AND PERCENTAGE OF PROSTATIC CANCER – POSITIVE REPEATED BIOPSIES: CROSS-SECTIONAL STUDY

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KORELATIVNA ANALIZA IZMEĐU BROJA UZETIH ISEČAKA PROSTATE I PROCENTA DIJAGNOSTIKOVANOG KARCINOMA PROSTATE U PONOVLJENIM BIOPSIJAMA: STUDIJA PRESEKA

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Received / Primljen: 17. 07. 2008. Accepted / Prihvaćen: 15.10.2008.

ABSTRACT

High-grade prostatic intraepithelial neoplasia (HGPIN) is the most important predecessor of prostatic adenocarcinoma (PA), and frequently co-exists with PA. The aim of our study was to correlate percentage of HGPIN discovered at initial biopsy and percentage of PA-positive repeated biopsies with the number of initial biopsy specimens and their morphology.

Our case series consists of 254 patients with initial needle biopsies of the prostate. The patients were divided into four groups according to the number of biopsy specimens: group I, with 2-3 specimens (69 pts); group II, with 4-5 specimens (47 pts); group III, with 6-7 specimens (99 pts); and group IV, with 8-10 specimens (39 pts). PA was found in 101 cases (39.8%) and HGPIN in 98 cases (38.6%). In 28 patients with PA (out of 101 cases), HGPIN was co-existent with PA (27.7%). PA was found in 18 cases among the patients from group I (26.1%), and HGPIN in 17 cases (24.6%) from the same group. In group II, PA was found in 16 cases (34.0%) and HGPIN in 17 cases (36.2%). In group III, PA was found in 46 cases (46.5%), and (47.5%) HGPIN in 47 cases. In group IV, PA was found in 19 cases (48.7%), and HGPIN in 20 cases (51.3%). In 35 out of 98 patients (35.7%) with HGPIN, repeated biopsies were performed. Among 13 cases with HGPIN found in one biopsy specimen only, repeated biopsy discovered PA in three of them (23.1%). Among 14 cases with HGPIN found in two biopsy specimens, repeated biopsy discovered PA in six of them (42.9%). Finally, among 8 patients with HGPIN found in 3 initial biopsy specimens, repeated biopsy found PA found in four of them (50.0%). Among 35 cases of HGPIN at initial biopsy, the most common histological types were tufting (60%) and micropapillar (25.7%), while the cribriform type was found in 11.4% of specimens, and the flat type in 2.9%. After repeated biopsy, PA was found the most frequently in patients with micropapillar (44.4%) and tufting (38.1%) types.

There was a significant correlation between the number of detected PAs and either the total number of specimens or the number of specimens with HGPIN at initial biopsy. Significant correlation was also found between tufting/micropapillar type of HGPIN and PA.

Key Words: high grade prostatic intraepithelial neoplasia, prostatic adenocarcinoma, biopsy of prostate.

SAŽETAK

Visokostepenoj prostatičnoj intraepitelnoj neoplaziji (VSPIN) je posvećena izuzetna pažnja kao najvažnijem prekursoru adenokarcinoma prostate (AP) i signifikantnoj udruženosti sa AP. Cilj istraživanja je bio da se uradi uporedna analiza VSPIN nađenog na inicijalnoj biopsiji i AP dijagnostikovanog na ponovnoj biopsiji u odnosu na broj uzetih isečaka i morfolo{ki izgled.

Analizirano je 254 pacijenta sa urađenom iglenom biopsijom prostate. Primenjene su patohistološke metode istraživanja. Pacijenti su na osnovu broja uzetih iglenih biopsija prostate svrstani u četri grupe: I grup 2-3 uzoraka (69 sl.), II grupa 4-5 (47 sl.), III grupa 6-7 (99 sl.) i IV grupa 8-10 uzoraka biopsije (39 sl.). U 101 sl. (39.8%) nađen je AP, a VSPIN u 98 sl.. (38.6%). U 28/101 sl. (27.7%) AP, nađen je udružen VSPIN. U I grupi AP je nađen u 18 sl. (26.1%) i VSPIN u 17 sl. (24.6%). U II grupi AP je nađen u 16. sl. (34.0%) i VSPIN u 17 sl. (36.2%). U III grupi AP je nađen u 46 sl. (46.5%), a u 47 sl. (47.5%) VSPIN. U IV grupi AP je nađen u 19 sl. (48.7%) i VSPIN u 20 sl. (51.3%). U 35/98 sl. (35.7%) sa VSPIN urađena je rebiopsija. Kod 13 sl., sa jednim isečkom zahvaćenim VSPIN, u rebiopsiji je u 3 sl. (23.1%) nađen AP. Kod 14 sl. sa 2 isečka zahvaćenim VSPIN, u rebiopsiji je nađen AP u 6 sl. (42.9%), i kod 8 sl. sa 3 isečka zahvaćenim VSPIN, u rebiopsiji je nađen AP u 4 sl. (50.0%). Od 35 sl. VSPIN na inicijalnoj biopsiji najčešći histološki tip bio je resičasti (60%) i mikropapilarni (25.7%), a kribriformni tip je nađen u 11.4% i pljosnat u 2.9%. Na rebiopsiji AP je najčešće nađen kod mikropapilarnog (44.4%) i resičastog tipa (38.1%).

Postoji značajna korelacija u detekciji VSPIN i AP u odnosu na broj uzetih isečaka i broj isečaka zahvaćenih VSPIN na inicijalnij biopsiji i na rebiopsiji, kao i korelacija između histološkog resičastog/mikropapilarnog tipa VSPIN prema AP.

Ključne reči: Visoko stepeni PIN, adenokarcinom prostate, biopsija prostate.

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INTRODUCTION

Prostatic adenocarcinoma (PA) is one of the most important human cancers, with rising incidence. It is more frequent in men of advanced age (1, 2, 3). Among men, PA is the cancer with the highest incidence, and the second most common cause of death from malignant diseases (after lung cancer). The mortality rate from PA is constantly rising (2).

According to epidemiological, histological, molecular and experimental studies, High Grade Prostatic Intraepithelial Neoplasia (HGPIN) is the most significant predictor of PA; it is considered to be the pre-malignant phase of PA (1, 3, 4). Four histological types of HGPIN have been described: tufting, micropapillar, cribriform and flat (1). Some studies have shown the high predictive value of HGPIN, which precedes PA by ten years (5, 4, 6, 7). Once discovered in a biopsy specimen, isolated HGPIN suggests the necessity for a clinical follow-up of the patient, including repeated biopsy of the prostate (8, 3, 6).

Some of the studies have shown that the detection rate of malignant lesions in the prostate increases with the number of specimens taken (9, 10, 11). Additionally, the correlation between the number of HGPIN-positive specimens taken at initial biopsy, their histological type and detection rate of PA at repeated biopsies was demonstrated (9, 12). On the other hand, there are studies that failed to show such a correlation (13). Due to these controversies and a relatively small number of studies in this area, further research efforts are necessary (13, 9, 6).

Our primary objective was to investigate the correlation between the detection rate of HGPIN and PA, and the number of tissue specimens taken by needle biopsy of the prostate.

Our secondary objective was to investigate correlations between the number of HGPIN-positive initial biopsy specimens or the incidence of certain histological types of HGPIN at initial biopsy, and the detection rate of PA at repeated biopsies.

MATERIALS AND METHODS

We have included 254 patients of the Clinical Centre "Kragujevac", Kragujevac, Serbia in our study. All the subjects received needle biopsies of the prostate between January 1st, 2003 and June 1st, 2007.

The tissue specimens obtained by needle biopsy of the prostate were fixed in 4% buffered formalin, inserted into paraffin blocks and cut by microtome to preparations 4-6 micrometers thick. Both standard haematoxylin-eosin and histochemical (Alcian blue, Masson) staining methods were used. The results were compared by Chi-square test.

The tissue specimens of the prostate (2-10 per patient) were taken by a needle from both the right and left lobes of the gland. According to the number of tissue specimens taken, the patients were classified into four groups: group I with 2-3 specimens (69 pts), group II with 4-5 specimens (47 pts), group III with 6-7 specimens (99 pts) and group IV with 8-10 specimens taken (39 pts).

In groups III and IV, 46 and 31 biopsies, respectively, were taken with the aid of transrectal ultrasound imaging.

Among the 98 patients with an HGPIN-positive initial biopsy, thirty-five were subjected to repeated biopsy of the prostate. The remaining 53 patients with HGPIN were lost to follow-up. According to the number of HGPIN-positive specimens at the initial biopsy, the patients were classified into three groups: group I – one HGPIN-positive specimen, group II - 2 HGPIN-positive specimens and group III – three HGPIN-positive specimens. The results were compared by student's T-test for independent samples.

RESULTS

Among the 254 patients subjected to needle biopsy of prostate in our study, one of the following clinical signs was always present: positive digital rectal examination of the prostate (DRE), elevated PSA or suspicious transrectal ultrasound imaging of the prostate.

The highest percentage of the study sample (Figure 1) consisted of patients in their eighth (123 pts, or 48.0%) or seventh (88 pts, or 34.7%) decade of life. Thirty-one patients (12.2%) were in their sixth, and 13 patients (5.1%) were in their ninth decade of life.

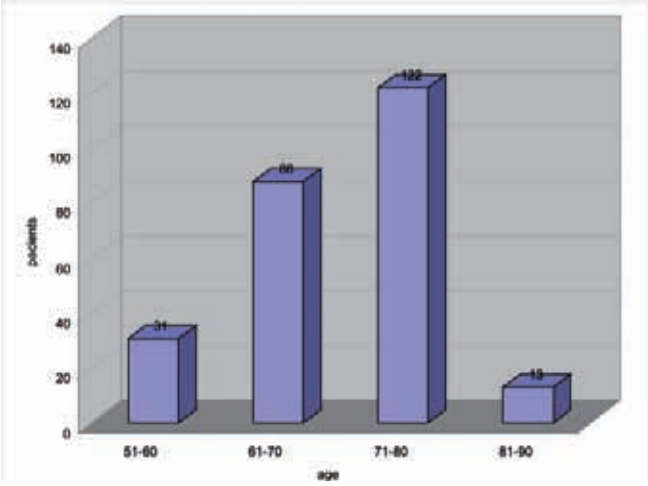

Figure 1. Age distribution of the study patients.

Table 1 shows the histological findings of the study samples. One hundred and one patients, with average age of 70.3 years, had PA (39.8%). Pre-malignant lesions were found in 117 patients (46.1%), and 98 of

them had HGPIN (38.6%, average age 69.5 years). The 28 patients with PA (101) had concomitant HGPIN (27.7%).

Table 1. Histological lesions from needle biopsies of prostate in 254 patients.

Histological lesions	No cases	%
Prostatic adenocarcinoma	101	39.8
HGPIN	98	38.6
Atypical adenomatous hyperplasia	13	5.1
Atypical small acynar proliferation	6	2.4
Florid hyperplasia of basal cells	7	2.8
Clear cells cribriform hyperplasia	3	1.2
Benign prostatic hyperplasia	64	25.2

Atypical adenomatous hyperplasia (AAH) was found in 13 cases (5.1%), and atypical small acynar proliferation (ASAP) in 6 cases (2.4%). Sixty-four patients (25.2%) had benign hyperplasia of the prostate (BHP), seven patients (2.8%) had rich hyperplasia of basal cells and there was bright cellular cribriform hyperplasia in three cases (1.2%).

Table 2 shows the comparison of detection rates of PA and HGPIN with the number of prostate tissue specimens taken at initial biopsy. In the group of 69 patients with 2-3 specimens, PA was found in 18 cases (26.1%) and HGPIN in 17 cases (24.6%). In the group of 47 patients with 4-5 specimens, PA was found in 16 cases (34.0%), and HGPIN in 17 cases (36.2%). In the group of 99 patients with 6-7 specimens, in PA was found46 cases (46.5%), and HGPIN was found in 47 cases (47.5%). In the group of 39 patients with 8-10 specimens, PA was found in 19 cases (48.7%) and HGPIN was found in 20 cases (51.3%). It is obvious from the table that the detection rates of PA and HGPIN increase with the number of tissue samples taken ($p<0.05$). However, the detection rates are similar in the groups with 6-7 and 8-10 tissue samples per biopsy ($p>0.05$).

Table 2. Comparison of detection rates of PA and HGPIN with number of prostate tissue specimens taken at initial biopsy.

Number of prostate tissue specimens	No cases 254	%	HGPIN – 98		PA - 101	
			No of cases	%	No of cases	%
2-3 specimens	69	27.2	18	26.1	17	24.6
4-5 specimens	47	18.5	16	34.0	17	36.2
6-7 specimens	99	39.0	46	46.5	47	47.5
8-10 specimens	39	15.3	19	48.7	20	51.3

In the patients with HGPIN, repeated biopsy of the prostate was infrequently performed. Out of 98 HGPIN-positive patients at initial biopsy, only 35 were subjected to repeated biopsy (35.7%), in 29 cases one repeated biopsy was done, and in 6 cases two, in a 6-12 month interval. The detection rate of PA at repeated biopsy was 37.1% (13 cases out of 35).

Table 3 shows the correlation between the number of HGPIN-positive initial biopsy specimens and the detection rate of PA at repeated biopsies. In the group of 13 patients with one HGPIN-positive specimen, repeated biopsy detected PA in 3 cases (23.1%). In the group of 14 patients with two HGPIN-positive tissue specimens, repeated biopsy detected PA in 6 cases (42.3%; 5 cases in the first repeated biopsy and 1 case in the second repeated biopsy). In the third group of 8 patients with 3 HGPIN-positive tissue specimens, repeated biopsy detected PA in 4 cases (49.0%; 3 cases in the first repeated biopsy and 1 case in the second repeated biopsy). Out of 13 patients in total with PA detected by repeated biopsies, eleven patients were subjected to one (84.6%) and two patients (15.4%) to two biopsies. There are significant differences in the detection rate of PA at repeated biopsies among the groups with different number of HGPIN-positive initial biopsy specimens ($p<0.05$).

Table 3. Correlation between number of HGPIN-positive initial biopsy specimens and detection rate of PA at repeated biopsies.

Number of HGPIN-positive needle biopsies	No of cases	The first repeated biopsy with PA		The second repeated biopsy with PA	
		No of cases	%	No of cases	%
One	13	3	23.1	0	0
Two	14	5	35.7	1	7.2
Three	8	3	37.5	1	12.5

Table 4 shows the comparison of histological subtype of HGPIN with the detection rate of PA at repeated biopsies. Among the 35 cases of HGPIN that were rebiopsied, the most frequent histological type was tufting (21/35 pts, or 60%), followed by micropapillar (9/35 pts, or 25.7%). Cribriform type was found in 4/35 cases (11.4%), and flat type in only one case (2.9%). After repeated biopsy, PA was the most frequent in the micropapillar (4/9 pts, or 44.4%) and tufting type (8/21 pts, or 38.1%). For the cribriform type, PA was found in 1/4 cases (25%). However, due to the small number of cases in each of the histological groups, the observed differences were not significant.

Table 4. Comparison of histological subtype of HGPIN and detection rate of PA at repeated biopsies.

HGPIN – 35 cases.			PA at repeated biopsy – 13 cases	
Histological type	No of cases	%	No of cases	%
Tufting	21	60.0	8	38.1
Micropapillar	9	25.7	4	44.4
Cribriform	4	11.4	1	25.0
Flat	1	2.9	0	0.0

DISCUSSION

The detection rate of HGPIN after needle biopsy of the prostate is higher than 25%, increases with age and is associated with PA in 85% of patients (3). In a large study by Cheng (3), presence of HGPIN at initial biopsy bears a risk of 35% for detection of PA at repeated biopsies. Therefore, detection of HGPIN at initial biopsy is an indication for repeated biopsy, especially if the patient is in good general health. Lowe (2) has shown an incidence of 36% during a 6-year study period. Schoenfirld (14) has followed 100 patients, detecting PA in 39%, HGPIN in 22% and associated PA and HGPIN in 26% of patients. After repeated biopsy in patients with isolated HGPIN (22 pts), PA was detected in 34% of them. In the same study, a higher number of HGPIN-positive tissue specimens were associated with a higher detection rate of PA at repeated biopsy.

HGPIN is associated with PA, and the incidence of HGPIN is higher in patients with PA, compared with healthy patients. Davidson (4), in his study of 100 patients with HGPIN and 112 patients without HGPIN, has detected prostate cancer in 35% of repeated biopsies in the first group, and in 13% of repeated biopsies in the second group. Other studies have had similar results (8, 6). All of these studies recommend strict follow-up of the patients with an HGPIN-positive initial biopsy. Kronz et al. (6) had analysed 245 patients with HGPIN-positive initial biopsies; in this group, repeated biopsy detected PA in 32.2% of cases, which is about one-third of all patients.

The results of our study fit well with already published data. One-hundred-and-one patients were PA-positive (39.8%), with an average age of 70.3 years. In total, premalignant lesions were found in 117 patients (46.1%), and HGPIN was found in 98 patients (38.6%), with an average age of 69.5 years. Additionally, in our study, HGPIN was significantly associated with PA. Twenty-eight patients with PA (27.7% of 101 patients) also had HGPIN. On the other hand, 35 patients with an HGPIN-positive initial biopsy (out of 98 patients in total) were subjected to repeated biopsy, and PA was found in 13 cases (37.1%). Therefore, the total association rate of HGPIN with PA in our study was 41/114 patients (36.0%). However, one should bear in mind that only 35.7% of patients with HGPIN were subjected to repeated biopsy, which is an unacceptably low rate.

According to other studies (10), the detection rate of PA increases with the number of tissue specimens taken. The detection rate of PA is higher with 2 or more biopsy specimens containing HGPIN (11, 15, 16).

Roscigno and associates (12) followed 47 patients with an HGPIN-positive initial biopsy for 11.4 months on average, and repeated biopsies (average number of biopsy specimens was 11.5); PA was detected at repeated biopsy in 21 patients (44.6%).

In our study, the detection rate of PA or HGPIN increased with the number of tissue samples taken ($p < 0.05$). The highest detection rate was observed in groups with 6-7 and 8-10 tissue specimens.

Roscigno (12) found that PA is more frequent in patients with multi-focal HGPIN (70%) than in patients with mono-focal HGPIN (10%). The patients with 10 to 12 tissue specimens at initial biopsy, with mono-focal or multifocal HGPIN, have a 45% detection rate of PA at repeated biopsy. Therefore, multi-focal HGPIN at initial biopsy is highly predictive for prostate cancer (14, 17, 18).

Abde-Khalek and associates (8) tried to find other significant predictors of PA among HGPIN-positive patients. Eighty-three patients with previous HGPIN-positive sextant biopsies (number of tissue specimens was 11) were followed, and the biopsies were repeated in 31 of them. Enrollment criteria for the second biopsy were an increase in PSA and/or abnormal findings from a digital rectal examination. The repeated biopsies detected PA in 30/83 patients (36%). The PSA level, digital rectal examination findings and transrectal ultrasound examination were not useful predictors of PA. However, the patients' age, PSA density and number of HGPIN-positive tissue specimens were independent predictors of PA ($p<0.001$)

Kronz and associates (6) have shown that the only independent histological predictor of PA was the number of HGPIN-positive tissue specimens: the cancer risk was 30.2% in patients with 1-2 specimens, 40% in patients with 3 specimens and 75% in patients with more than three HGPIN-positive tissue specimens. Bishara and associates (9) followed 132 patients with an HGPIN-positive initial biopsy, and found PA at repeated biopsy in 28.8% of patients (89.5% were detected at the first two repeated biopsies). The patients with 2 or more HGPIN-positive tissue specimens at initial biopsy had PA at repeated biopsy in 35.9% of cases, and those with only one HGPIN-positive specimen had PA in 22% of cases. In total, PA was detected in 32% of patients with an HGPIN-positive initial biopsy. Therefore, the patients with multiple HGPIN-positive tissue specimens at initial biopsy bear a highest risk of PA.

Naya et al. (13) also investigated whether presence or number of HGPIN-positive tissue specimens at initial biopsy were predictors of PA at repeated biopsies. At initial biopsy, PA was detected in 33.8% of cases and HGPIN in 20.8%. Incidence of HGPIN among the patients with PA-positive initial biopsy was 29.7%. In 175 patients without PA, at least one biopsy was repeated (1 to 3, with an average interval of 3 months), and 47 were HGPIN-positive. In total, repeated biopsies detected PA in 18.3% of 175 patients. The number of initial biopsy tissue specimens was not associated with the probability of PA at repeated biopsy. The authors concluded that the number of HGPIN-positive tissue specimens was not predictive of PA. Scottoni et al. (17), in their recent publication, analysed the detection rate of PA in a much larger sample of patients, which were divided into groups with 12 or 18 tissue samples. There was not a significant

difference in the detection rate of PA among the groups (38.4% and 39.9%, respectively).

In our study, the number of HGPIN-positive tissue specimens at initial biopsy was associated with the detection rate of PA at repeated biopsy. In the group of 13 cases with one HGPIN-positive specimen, PA was detected in 23.1% of the first repeated biopsies. In the group of 14 cases with 2 HGPIN-positive specimens, PA was detected in 42.3% of repeated biopsies. In the group of 8 cases with 3 HGPIN-positive specimens, PA was detected in 49.0% of repeated biopsies. Out of 13 total patients with PA detected at repeated biopsies, eleven patients were subjected to one (84.6%) and two patients (15.4%) to two biopsies. The detection rate of PA at repeated biopsy increases with the number of HGPIN-positive tissue samples at initial biopsy ($p<0.05$).

Bishara and associates (9) stressed the association between isolated HGPIN and invasive prostatic cancer. They investigated whether histological types (tufting, micropapillar, cribriform or flat) of HGPIN or the number of HGPIN-positive tissue specimens at repeated biopsy were predictive of a higher risk of invasive prostatic cancer. There were 200 HGPIN-positive needle biopsies (average patient age was 66.4 years). The tufting type was present in 59% of cases, micropapillar in 34.3%, cribriform in 6.2% and flat in 0.5% of cases. The tufting and flat types were associated with PA at repeated biopsies in 31.9% of cases, while micropapillar and cribriform types were associated with PA in 22% of cases. They concluded that histological type is more than simply an informative characteristic.

Kronz et al. (6) has shown the following histological parameters from initial biopsy to be predictive of PA: presence of mitoses, number of HGPIN-positive tissue specimens, predominantly micropapillar or cribriform HGPIN and presence of a large, prominent nucleolus.

Among the 35 cases of HGPIN that were re-biopsied, the most frequent histological type was tufting (21/35 pts, or 60%), followed by micropapillar (9/35 pts, or 25.7%). Cribriform type was found in 4/35 cases (11.4%), and flat type was found in only one case (2.9%). After repeated biopsy, PA was most frequent in the micropapillar (4/9 pts, or 44.4%) and tufting types (8/21 pts, or 38.1%). There is stronger association between the tufting/micropapillar type and PA than between the cribriform/flat type and PA. However, the tufting and micropapillar types were the most prevalent, and the total number of repeated biopsies was small.

The results of our study suggest that the patients with multiple HGPIN-positive tissue specimens at initial biopsy bear the highest risk of invasive PA.

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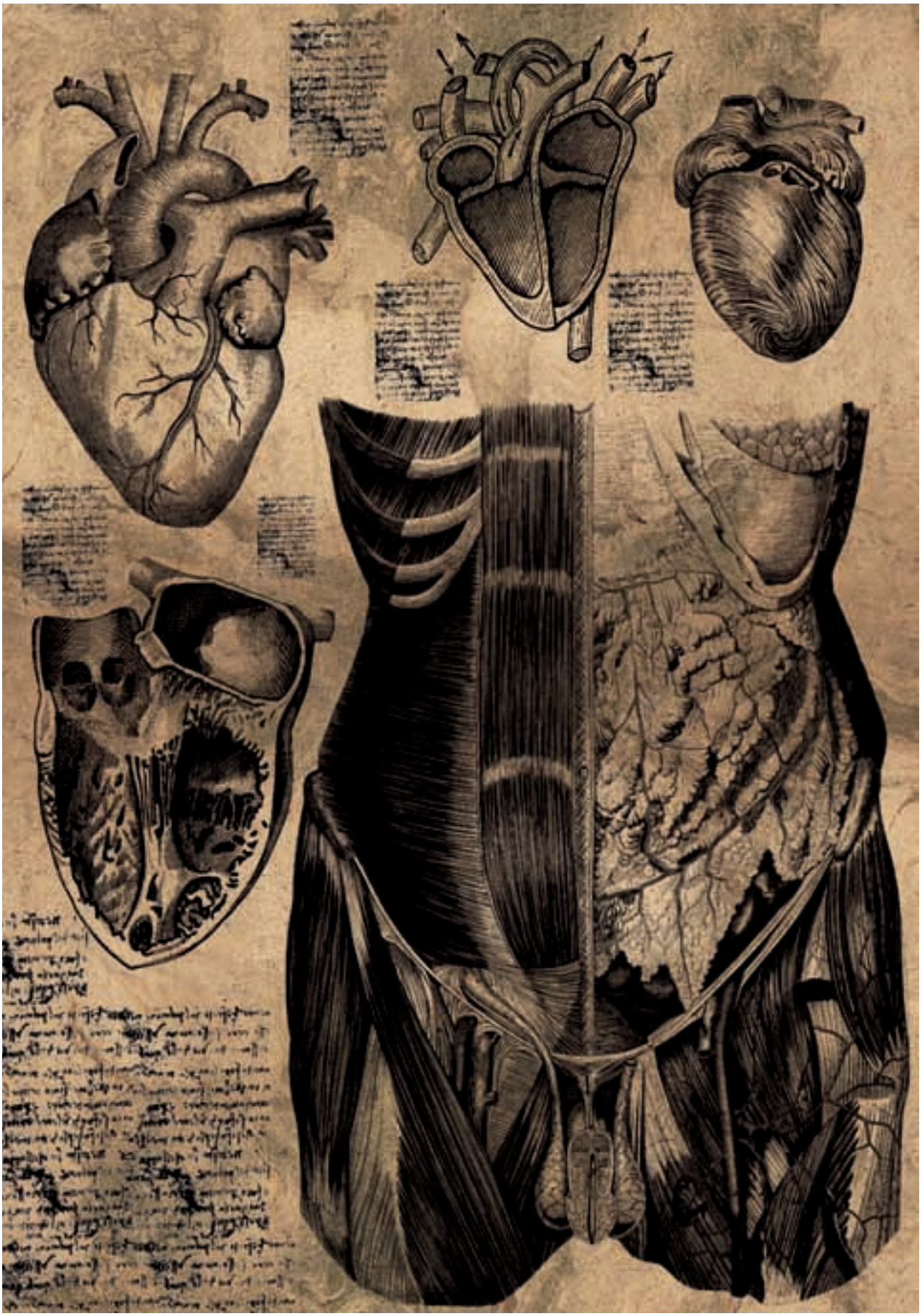

UDK 618.14-006.31-07 / Ser J Exp Clin Res 2008; 9 (3): 99 – 103

A COMPARATIVE STUDY OF LAPAROSCOPIC AND ABDOMINAL HYSTERECTOMIES

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KOMPARATIVNA STUDIJA LAPAROSKOPSKE I ABDOMINALNE HISTEREKTOMIJE

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Received / Primljen: 19. 06. 2008. Accepted / Prihvaćen: 15.10.2008.

ABSTRACT

This case study shows comparative results of laparoscopic and abdominal hysterectomies. It includes 503 hysterectomies in connection to myomas of the uterus, performed in the period between January 2000 and December 2006. There were 64 (12.75%) patients that underwent laparoscopic hysterectomy (LH) and 439 (87.28%) patients subjected to abdominal hysterectomy (AH). The average age of patients subjected to LH was 48.91 years; for those in the AH group, average age was 46.47 years (P<0.01). Statistically speaking, there was a considerable difference between AH and LH in the number of myomas (2.06 vs.2.90), volume (281.0373 cm^3 vs. 476.9426 cm^3) and weight of the uterus (236.25 grams vs. 431.53 grams). The average duration of surgical procedures for LH was 98.8 minutes, while the AH procedures lasted for an average of 67.52 minutes (p<0.01). The main advantages of laparoscopic interventions include minor deterioration in the quality of blood test results and fewer patients demanding a blood transfusion. The overall cost benefit also speaks in favour of the laparoscopic procedure.

Key words: laparoscopic hysterectomy, abdominal hysterectomy

Main objective: To show comparative differences between the two methods elucidating their main advantages and disadvantages

SAŽETAK

U radu su prikazani uporedni rezultati između dve vrste histerektomije: laparoskopske i abdominalne.U periodu od januara 2000 godine do decembra 2006 godine urađene su 503 histerektomije radi mioma uterusa.U grupi laparoskopskih histerektomija (LH) bilo je 64 (12,75 %) a u grupi abdominalnih histerektomija (AH) bilo je 439(87,28%) pacijenta.Prosečna starost pacijenata u grupi pacijenata kod kojih je rađena laparoskopska histerektomija bila je 48,91 a u grupi pacijenata operisanih na klasičan način iznosila je 46,47 godine (p<0.01).Postoji statistički značajna razlika po broju mioma (2,06 vs 2,90),volumenu (281,0373 vs 476,9426 cm^3) i težini uterusa (236,25 vs 431,53 gr.) odstranjenih klasičnim, abdominalnim putem u odnosu na drugu grupu operisanih pacijenata. Prosečna dužina trajanja operacije kod laparoskopskih operacije je 98,8 min.a kod abdominalne histerektomije 67,52 (p<0,01) .Manji pad krvne slike je posle laparoskopske histerektomije i ne postoji statička razlika u broju pacijenata koji su primili transfuziju. Takođe i cost benefit je na strani pacijenata koji su operisani laparoskopski.

INTRODUCTION

In almost 90% of cases, hysterectomy is the preferred treatment option for women suffering from symptomatic myomas. It is the most frequent gynecological operation. Depending on the size of the uterus and location of the myoma, (as revealed by gynecological examination and ultrasound or some alternate diagnostic procedure), hysterectomies can be abdominal, vaginal or laparoscopic. Determining the parameters for a particular hysterectomy, in order to provide satisfactory results, requires accurate diagnostics and pathohistological examination. Although 77% of females have myomas (1, 2, 3, 4), almost 80 % of myomas are asymptomatic (1) and do not require medical treatment. According to ACOG results from 2000, indications for hysterectomy comprise myomas of the uterus (33.3%), endometriosis (18.8%), prolapse of the uterus (16.4%), carcinomas (10.1%), endometrial hyperplasia (5.3%) and other causes (15.9%) (1).

The number of hysterectomies in the USA has not changed significantly in recent years, but the variety of hysterectomies has diversified. The percentage of abdominal hysterectomies has decreased from 73.6% to 63%, while the number of laparoscopic interventions went up from 0.3% to 9.9%. The percentage of vaginal

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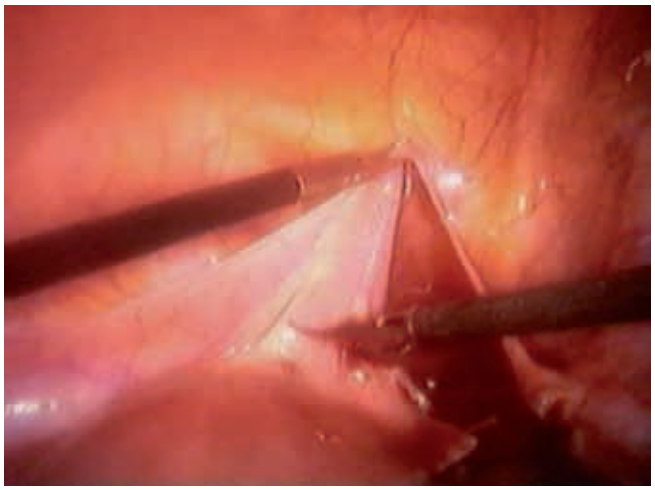
hysterectomies has stayed approximately the same (24% to 23.3%). Similar results were also confirmed by a Finnish study over the span of 8 years. In 1990, abdominal hysterectomies accounted for 93% of all procedures; vaginal hysterectomies were performed in 7% of cases; there were no laparoscopic interventions. Only eight years later (in 1998), 45% of hysterectomies were abdominal, 27% were vaginal and 28% were laparoscopic hysterectomies (5, 6, 7).

The rapid decrease in the frequency of standard hysterectomies in comparison to laparoscopic procedures is influenced by the development of a new, minimally invasive surgical procedure.

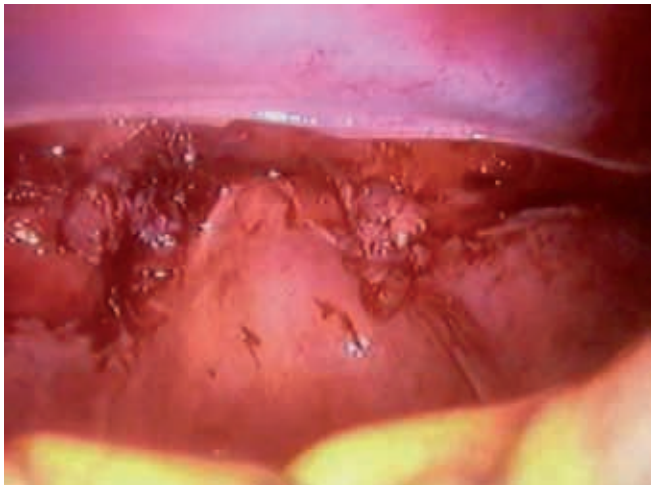
LAPAROSCOPIC HYSTERECTOMY

The first laparoscopic hysterectomy (LH) was performed by Harry Reich on January 2, 1988 (9,10). It was followed by numerous experimentations with technique, varying according to which part of the operation was performed using laparoscopic vs. vaginal methods (11, 12, 13, and 14). This combination of different approaches was aimed at providing patients with an optimal level of comfort and minimizing possible health hazards.

Operating instructions: After the physician has inserted the Veres needle, patients are insufflated with approximately 3 to 4 liters of carbon dioxide, leveling the intra-abdominal pressure at 15 to 17 mm Hg. This inflation facilitates satisfactory distension of the abdominal wall and does not hinder the venous circulation of the abdomen or pelvis minor. The amount of CO_2 insufflated and the intra-abdominal pressure depend on the physical constitution of the patient. Subsequently, pneumoperitoneum is formed in the umbilical area, which is then used to insert a trocar bearing optical devices and a camera, while the patient is placed in a Trendelenburg position at a 60° angle (16), in order to provide access to the pelvis minor. The incision and coagulation are performed using 35W bipolar, high-frequency, low-voltage electrical current. Bipolar current secures minimal thermal expansion to the surrounding tissue and prevents undesired side effects. The vagina is opened by means of monopolar current, or scissors in combination with bipolar coagulation. Upon opening the vagina, there is a gas outflow and the frontal abdominal wall drops down. After removing the desired specimen vaginally, a few individual stitches are made and the pneumoperitoneum is then recreated in order to scope the entire operating field. If necessary, an additional haemostasis is performed and then the whole operating field is rinsed with 2 to 3 litres of Ringer's solution, in order to neutralize negative allergic reactions of the peritoneum caused by carbonic acid created during the re-absorption of CO_2. If necessary, a draining tube is inserted into the Douglas cave. Insertion points on the skin are repaired by means of Dexon stitches. If the trocar is 10mm wide, the fascia is also closed.



Picture 1. Opening plica vesico-uterina



Picture 2. Operatively field after

ABDOMINAL HYSTERECTOMY

Abdominal hysterectomy is performed using general or localized anesthesia, by means of medial or suprapubic Pfannenstiel incision. The type of laparotomy depends on the physical constitution of the patient, size of the uterus, auxiliary diagnoses, surgeon experience and esthetic demands of the patient. The removal of the uterus can be performed extra-fascially according to Richardson (standard), or inter-fascially according to Aldridge (21).

Myomatosis of the uterus requires one of three basic hysterectomies:

1. Total abdominal hysterectomy (TAH);
2. Subtotal (supracervical) hysterectomy;
3. Total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BSO)

Upon removing the uterus (in the case of TAH), the vagina is closed using individual or continuous stitches. With

subtotal hysterectomy, the remaining part of the uterine cervix is stitched with continuous or individual stitches.

RESULTS

In the period between January 2000 and December 2006, 503 hysterectomies were performed in order to remove uterine myomas. Of these, 64 procedures (12.75%) were laparoscopic and 439 (87.28%) were abdominal hysterectomies. The average age of patients subjected to LH was 48.91 years; the average age of those in the AH group was 46.47 years ($p<0.01$).

Table 1: A comparison of the number of myomas and size of the uteri in the two study groups

	Laparoscopy	Abdominal	p
N°. of myomas	2.06	2.90	p<0.05*
Weight (grams)	236.25	431.53	p<0.01**
Volume (cm^3)	281.0373	476.9426	p<0.01*

Statistically speaking, there was a considerable difference between the AH and LH when the number of myomas, volume and the weight of the uterus was concerned.

Table 2: Postoperative hematological parameters and the number of patients receiving transfusion

	Laparoscopy	Abdominal	p
Postoperative Er	3.56	3.53	NS
Postoperative Hg	10.29	9.65	p<0.01**
Postoperative Hct	31.54	30.53	NS
Postoperative MCV	84.18	82.32	p<0.05*
Transfusion	59 (92.2%)	354 (84.3%)	p>0.05

These results show that there was a statistically significant difference in Hg and MCV values between LH and AH groups. However, the Er, Hct and the number of blood transfusions showed no significant difference in the two control groups.

Table 3: Operation duration and cost benefit parameters

	Laparoscopy	Abdominal	p
Operation duration	96.80	67.52	p<0.01**
Antibiotics	9 (14.1%)	206 (46.9%)	p<0.01**
Surgical thread	7.9	2.42	p<0.01**
Infusion solution	1492.19 ml	6835.99 ml	p<0.01
Hospitalization	3.33	5.84	p<0.01
Duration of sick leave	14.44	38.40	p<0.01

Average duration of surgical procedures for LH was 98.8 minutes, while the AH procedures averagely lasted for 67.52 minutes ($p<0.01$). Cost benefit analysis shows a significant difference related to the amount of therapeutic use of antibiotics, thread material, postoperative infusion expenditure, hospitalization and sick leave duration in favour of LH.

Table 4: Types of postoperative complications

Type of complication	LH	AH
Lesions of the uterus	1	-
Postoperative bleeding	1	-
Ileus	1	1
Peritonitis	-	2

LH group showed three cases of postoperative complications: a lesion of the uterus, postoperative bleeding 2 hours after the procedure and subileus occurring on the third postoperative day. None of the complications were diagnosed during the procedures and all of them were treated by means of laparatomy. AH group also presented three cases of complications: one ileus and two complete dehiscences of the frontal abdominal wall caused by infections of the peritoneum.

DISCUSSION

According to the relevant literature, the average age of patients subjected to laparoscopic hysterectomy is between 47.2 and 60.9 years (2, 16, 22, 23), which corresponds to the age of patients included in this study. For LH, the average patient age was 48.91 years (ranging from 32-67 years); for AH, the average patient age was 46.47 years (29 to 76 years). Both types of hysterectomies had similar indications, with contraindications occurring mainly for LH (9, 10, 24). The list of contraindications included cardio-pulmonary state of the patient, pelvis minor block**,** enormous obesity**,** postpartum bleeding and advanced endometrial carcinoma. Laparoscopic hysterectomy can also be contraindicated by the size of the uterus and the size, number and location of the myomas. Standard hysterectomy is performed on patients with a larger number of myomas and with heavier and larger uteri. Both techniques inevitably lead to the deterioration of blood samples. The accurate selection of patients suited for laparoscopic procedures results in less excessive bleeding. By analyzing postoperative values of Er, Hg, Htc and MCV, we can conclude that the decrease of Er and Htc is statistically higher with patients subjected to the standard procedure, while Hg and MCV show no significant difference. Although there is no statistical difference in the number of patients receiving postoperative transfusion, the table shows that 15.5% of AH patients received transfusions, in comparison with 7.8% patients from the LH group. Laparoscopic hysterectomy is technically more difficult for the surgeon to perform and easier for the patient to endure. The longer duration of the laparoscopic procedure is necessary due to: 1) no direct view of the operating field, 2) absence

of the third dimension, 3) absence of direct touch, 4) working in a limited area that does not allow proper handling of the uterus. Duration of the operation can be negatively influenced by insufficient experience of the performing surgeon. Alternatively, the size of the uterus (6) can have a positive effect on the duration of the procedure. When the laparoscopic technique was initially introduced, the average operation lasted between 2-4 hours (6, 7, 11). Operating time decreases with increasing surgeon experience and the use of staplers (6, 9). Abdominal hysterectomy has a significant advantage over laparoscopic hysterectomy in terms of time consumption (6, 12, 13, 16, 22). The average duration of a standard hysterectomy is 67.52 minutes (ranging from 40-140 minutes), which is significantly shorter than the laparoscopic procedure, lasting an average of 98.8 minutes (ranging from 45-140 minutes). The overall expenditure of surgical thread is almost three times higher with abdominal procedures (7.9 threads versus 2.4 threads in laparoscopic operations). Individual stitches for closing the vagina were used in 57.1% of abdominal and 78.4% of laparoscopic procedures. Individual stitches enable "cleansing" of the abdominal cavity from residual rinsing liquids and blood, as well as control of possible postoperative bleeding. This also prevents narrowing of the upper part of the vagina after it has healed completely, which is one of the most frequent postoperative causes of dyspareunia. For LH patients, strict adherence to the basic principles of microsurgery by surgeons and the ability to avoid excessive bleeding during the operation result in a significantly smaller postoperative consumption of antibiotics. As AH includes laparotomy, patients spend between 24 and 72 hours in the Intensive Care Unit, demanding four times more infusion than the LH patients, who leave the ICU not more than 24 hours after the operation. There are also significant differences in hospital stay and sick leave duration. Average hospital stay and sick leave of LH patients are 3.33 and 14.44 days respectively, while AH patients ask for 5.84 and 38.4 days. Among the patients receiving hysterectomies, there were six postoperative complications. The LH group of patients had three cases with complications. Firstly, there was thermal damage of the urethra entering the bladder, which led to necrosis and formation of a vesicovaginal fistula appearing on the tenth postoperative day. Secondly, a patient suffered postoperative bleeding of the back vaginal wall two hours after the operation. Finally, in one case, a spark induced by the monopolar instrument caused burns to the colon, which resulted in subileus forming on the third postoperative day. All aforementioned complications were dealt with using laparotomy. Considering that hysterectomy is deemed an advanced laparoscopic surgical procedure, this number of lesions can be considered quite high. According to information found in the literature, the number of complications occurring in laparoscopic hysterectomies ranges from 0.02 – 8.3:1000 operations (6) for urethra lesions, and 3.57:1000 operations for intestinal lesions. Injury to the urethra is more common in laparoscopic (relative risk 7.2) than in abdominal operations (14). Standard hysterectomy showed three cases of complications: one ileus and two complete dehiscences of the frontal abdominal wall caused by infections of the peritoneum.

Laparoscopic hysterectomy can certainly replace abdominal hysterectomy. In other words, it can turn abdominal hysterectomy into vaginal hysterectomy, which is much less invasive than the standard abdominal hysterectomy procedure. It is also the method of choice in cases where vaginal hysterectomy cannot be conducted due to a previous surgical intervention (abdominal or vaginal), endometriosis, adnexal structures, narrow vaginas of nulliparous patients and narrow pelvises. The laparoscopic procedure cannot be performed in approximately 20% of planned cases (25). Accurate diagnostics and pathohistological examinations of the tissue are of crucial importance when deciding on a particular method of hysterectomy, in order to provide the best possible results for the patient.

CONCLUSION

Laparoscopic hysterectomy is a good choice for accurately selected patients. It is characterized by a lower complication incidence, less postoperative pain, less of a need for preventive antibiotics, shorter period of hospitalization, less complicated postoperative care and faster return of patients to everyday life and activities: all factors leading to a more satisfied patient. Negative aspects of this type of hysterectomy are longer operation procedures and a slightly higher rate of urethra lesions. Increased surgical experience and development of new endoscopic equipment can lead to a decrease in lesion occurrence and shorter procedures. Even though laparoscopy is more suitable for smaller uteri, the development of surgical techniques, as well as extraction methods, will increase the applicability of the laparoscopy procedure.

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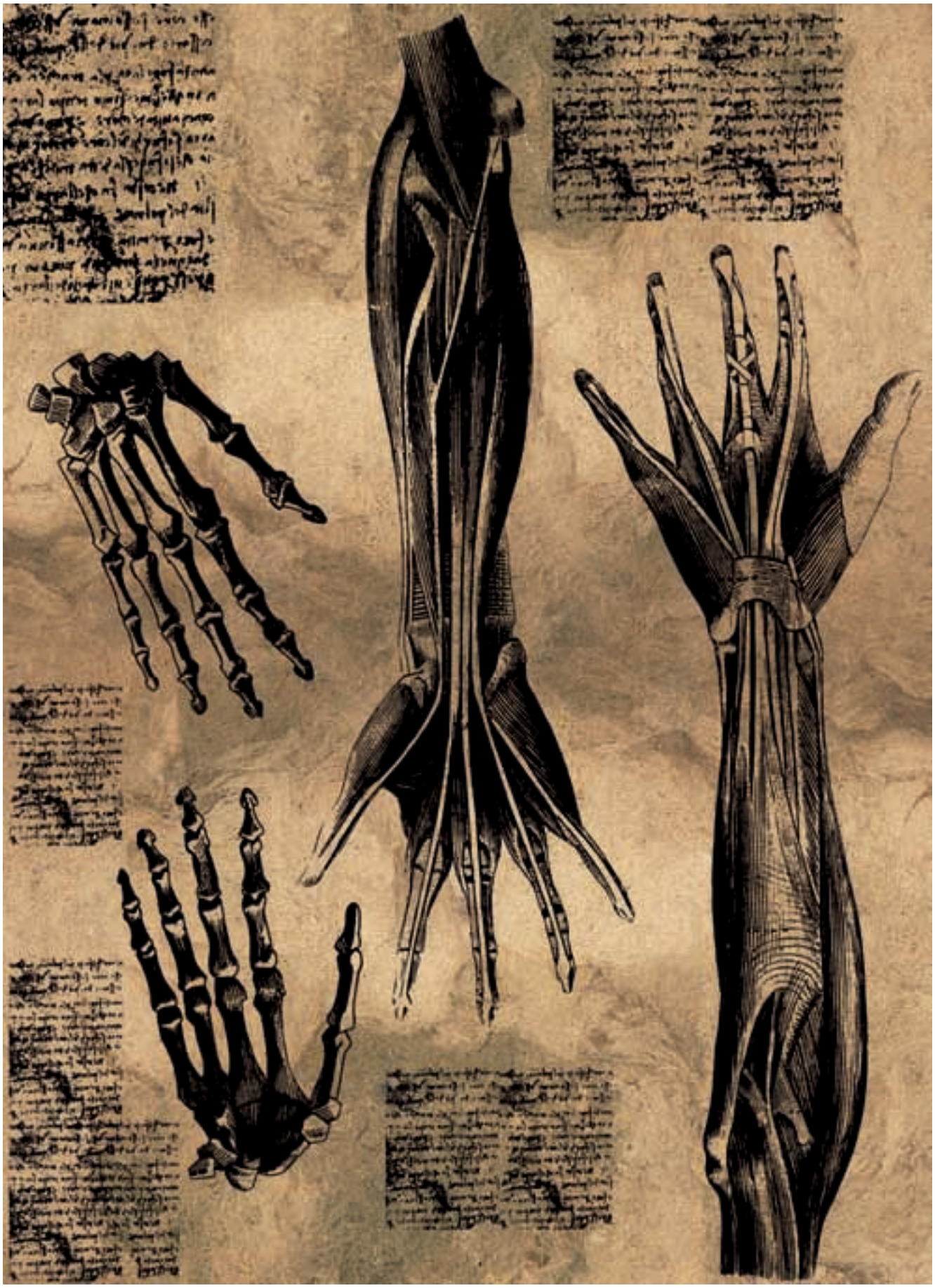

UDK 616.714-001 / Ser J Exp Clin Res 2008; 9 (3): 105 – 110

PROGNOSIS OF THE OUTCOME FOLLOWING SEVERE CLOSE CRANIOCEREBRAL INJURY

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PROGNOZA ISHODA TEŠKE ZATVORENE KRANIOCEREBRALNE POVREDE

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Received / Primljen: 20. 09. 2007. Accepted / Prihvaćen: 15.10.2008.

ABSTRACT

We describe the parameters that can influence the outcome of severe closed craniocerebral injury. Craniocerebral injuries involve two phases: the phase of primary injury, which includes primary cerebral lesions made under the influence of factors that have a direct or indirect impact on the cranium, and the phase that represents secondary injuries, which are the result of the impact of pathophysiological mechanisms (hypoxia, hypercapnia, and hypotension). These mechanisms are initiated by a trauma as well as some specific characteristics of the organism (e.g., age, comorbid conditions, and previous injuries). The aim of the study was to establish factors that significantly influence the outcome after severe closed craniocerebral injury. A total of 182 patients that were treated at the Clinical Center in Kragujevac from 2001 to 2005 were included. The most important prognostic factors for the treatment outcome were: age, oxygen saturation, the Glasgow Coma Scale score (GCS) and level of injury of the cerebral tissue diagnosed by a CT. Morbidity risk raised by 2.6% when only the age parameter increased by one year. When evaluating a 1% increase in oxygen saturation, the morbidity risk rate fell for 10.7% of the patients if other parameters remained stable. According to the aforementioned criteria, a 159.3% increase in morbidity risk rate was observed between mild and severe brain injury when other parameters did not change. In conclusion, a severe closed cerebrocranial injury is a dominant factor for the determination of patient outcome.

Key words: craniocerebral injury, outcome, neurosurgery

SAŽETAK

Ova studija obradjuje parametre koji mogu da utiču na ishod tehke kraniocerebralne povrede. Kraniocerebralne povrede imaju dve faze: fazu primarnih oštećenja koja uključuje primarnu cerebralnu leziju uzrokovanu faktorima koji imaju direktni ili indirekti uticaj na kranijum, i drugu fazu, koja predstavlja sekundarna oštećenja, nastala kompleksnim patofiziološkim mehanizmima (hipoksija, hiperkapnija, hipotenzija) i koja su inicirana traumom i nekim specifičnim karakteristikama organizma (starost, komorbiditeti, prethodne povrede, i dr.). Cilj studije je bio da utvrdi koji faktori najviše utiču na ishod. Ukupno je uključeno 182 bolesnika, koji su lečeni u Kliničkom centru Kragujevac, od 2001. do 2005. godine. Najznačajniji prognostički faktori terapijskog ishoda su bili: starost, saturacija kiseonika, skor Glazgov koma skale (GKS) i nivo oštećenja cerebralnog tkiva, dijagnostikovan CT-om. Za svaku godinu života povećavao se rizik morbiditeta za 2.6%, kada se drugi parametri nisu menjali. Povećanje saturacije kiseonika za 1% je smanjilo stopu morbiditeta za 10.7%, kada se drugi parametri nisu menjali. U pogledu cerebralnih tkivnih oštećenja (na osnovu rezultata CT-a), razlika izmedju lake i teške moždane povrede, prema prethodno definisanim kriterijumima, je povećala rizik morbiditeta za 159.3%, kada se drugi parametri nisu menjali. U zaključku, te{ka zatvorena kraniocerebralna povreda je dominantni faktor ishoda kod politraume.

Ključne reči: kraniocerebralna povreda, ishod, neurohirurgija

INTRODUCTION

Injuries of all kinds are a significant medical, economic and social problem. The injuries of the cranium and brain in overall traumas are very significant. They have an enormous influence on the life of an individual, on close and distant family members, as well as on society, because of their high incidence and the future difficulties they impose.

The annual incidence of head trauma is between 0.2 and 0.3% and is most common between the ages of 15 and 24. The risk decreases by the age of 50 but becomes more frequent in the elderly (1). Head trauma is the leading cause of death in the population under 44 years of age. In the population as a whole, the incidence of head trauma mortality is third after vascular (cerebrovascular and cardiovascular) and malignant diseases. Studies in Great Britain have shown that closed craniocerebral injuries had severe consequences in 91 out of 419 people per 100,000 citizens (2). In the USA,

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two million people suffer from cranial injury annually and, after the final treatment, 50,000 people suffer from severe consequences (3). According to the data from the USA, 500,000 people are hospitalised with closed cranial injuries annually, 15% of whom have severe craniocerebral injuries (4). The incidence of morbidity is estimated to be 25 per 100,000 citizens (1). In the USA, 56,000 people die from head trauma annually (5). According to the same sources, 20-40% of the total injuries are in traffic accidents and 2/3 of the patients die on the way to the hospital. According to data from Slovenia, 30,000 people were treated for different injuries in 2001 and 29% of these cases suffered from a head injury. In 2001, 570,000 people were treated for various injuries at the primary health care level, cranial injuries accounting for 13% of the cases (approximately 75,000 people).

The classification of craniocerebral injuries by severity is based on different criteria. All classifications are made according to a scheme. The most applied criteria are the Glasgow Coma Scale (GCS), coma duration, duration of post-traumatic amnesia, and the results of neuroimaging and electrophysiological diagnostic methods. All classifications are of theoretical, therapeutic and prognostic importance. None of the mentioned criteria are sufficient to show total and precise severity of the cranial and cerebral injury, as this is also influenced by age and other factors. In elderly people, even minor craniocerebral injuries can be complicated by haematomas and complications of other systems and organs, increasing the morbidity and making the prognosis less predictable. Previous cranial and brain traumas are also negative factors for prognosis. One previous injury doubles the risk of the trauma and two makes the risk eight times greater (6).

In practice, the most common criterion for the classification of severity of craniocerebral injuries is the GCS (7). According to this criterion, craniocerebral injuries are classified as a) mild (GCS score from 13-15), b) moderately severe (9-12) and c) severe (3-8). If craniocerebral injuries are defined by only the GCS, some mistakes can occur because craniocerebral injuries can change due to bleeding that can occur afterwards. The preserved state of consciousness does not guarantee that intracranial lesions, such as contusions or haematomas, will not appear. This can also change the clinical picture and significantly influence the prognosis after craniocerebral injury. Intoxication with alcohol or the use of sedatives in the traumatised patient is associated with lower GCS values that do not correlate with the severity of craniocerebral injury.

Research has shown that coma duration is correlated with the severity of injury and its outcome. Using this as a criterion, craniocerebral injuries can be classified as a) mild, where the coma lasts for 20 minutes (some authors include up to 30 minutes), b) moderately severe, where the coma lasts up to six hours, and c) severe, where the coma duration is over six hours from the point of hospitalization (6). Patients with moderately severe craniocerebral injuries suffer from many subsequent problems, such that two-thirds of the injured cannot be professionally rehabilitated (6).

In some studies, a significant part of the estimation of the severity of craniocerebral injury is post-trauma amnesiac duration. This mostly correlates with the GCS and lasts four times longer than the post-trauma coma (6). In practice, this fact is very unreliable due to many reasons, such as aphasia, the confused condition of a patient and the fact that the patients are released from hospital before the amnesiac period passes.

There are several issues regarding the definition of severe closed craniocerebral injury. Most authors agree that the group of severe closed craniocerebral injuries consists of the injuries with GCS score of 8 and lower. The pathological substrate in a severe craniocerebral injury is primarily the contusion of the cerebral hemispheres, the brain stem and diffuse axonal lesions.

Severe closed craniocerebral injury immediately after trauma is manifested as problems ranging from breathing disorders to apnea and disorders caused by bradyarrhythmia to arrest (8). Respiration disorders and apnea induce hypoxia and ischemia in the brain and lead to disturbances of consciousness. If these complications last for a long enough, they can have an adverse influence on the craniocerebral injury. The kinetic energy of mechanical devices that cause craniocerebral injury induces dysfunction of the respiratory centre in the medulla oblongata (9). Premature respiratory support in these cases is a prerequisite for a positive outcome.

The follow-up of a patient with a craniocerebral injury is of crucial importance. There are patients who feel very well between the injury and the moment of worsening. The term for this is “speak or vanish” or “speak and aggravate” (10). This typically occurs in patients with a GCS score of 13 or 14 who were injured while falling down. This is demonstrated by the rule that estimation of an initial condition can be incorrect. After recovering from a coma, these patients are anxious, distressed, disoriented in both time and space or towards people, depressive or euphoric, uncritical, nervous and hypermotoric (11); a condition called post-traumatic delirium. If the brain stem is damaged, dysarthria, unbalance, fatigue, ophthalmoplegia and rare pyramidal defects can be observed. Furthermore, post-traumatic amnesia can still be present (10). By new radiographical methods, we can identify the formation of a new bone in periarticular zones, which can mostly be seen in the proximal extremity of the wrists in over half of the patients who recovered from a coma (12). The precise mechanism of appearance of these changes is unknown, however, it is suspected that prolonged coma, muscle hypotonus, weakness of the extremities and fractures are risk factors.

It is of great interest to identify the factors that influence the outcome of closed craniocerebral injury. It is well known that closed craniocerebral injuries result in mortality in most cases, with a rate of over 45%, although

some studies suggest that the mortality rate is lower than 40%. Although these are studies from different regions and in different circumstances, the difference is lower than 10%. Premature death in the first 48 hours is a clear consequence of primary cerebral damage. Death occurring later is the consequence of various processes that are related to hypoxia, hypercapnia and hypotension. These factors can become more pronounced when there is a severe and heavy injury of other systems and organs. The aim of this study was to define risk factors for poor outcome in order to allow the introduction of novel, adequate therapeutic measures that could make the outcome of injury more favourable.

PATIENTS AND METHOD

We included patients treated at the Neurosurgical Department and Intensive Care Unit at the Clinical Centre in Kragujevac from January 1st, 2001 to December 31st, 2005. Patients had closed craniocerebral injuries (isolated or together with polytrauma) with GCS scores upon admission of 8 and lower. A total of 111 closed craniocerebral injury patients that had no other injuries were included, along with 71 patients with the severe closed craniocerebral injury coupled with the severe injury to other systems and organs (e.g., rib cage, spinal column, pelvis, abdominal organs or fracture of long bones of the extremities).

The design of the study was set up as previously described (13). We investigated socio-demographic data, anamnesis and heteroanamnesis variables, clinical status at admission, radiological imaging, biochemical tests, treatment modalities, and variables of clinical outcome including the outcome according to Glasgow outcome scale (GOSE).

For statistical analysis, a Kolmogorov–Smirnov test was used for evaluating variables within normal limits. Other tests were used with the appropriate methods that were consistent with the type and distribution of the original data (14). A t-test and Mann-Whitney test were used for comparison of two means. Frequency of the observations was tested by a chi-square test and Fisher's exact test. A multifunctional regression analysis was performed to test the relationship between two variables. Univariate and multivariate binary logistic regressions were used for testing the cause-and-effect relationship between treatment outcomes and other variables. The receiver operating characteristic (ROC) curve and area under the curve (AUROC) analyses were used for ensuring that a variable could be the test marker for a fatal outcome prediction (15).

RESULTS

During the study period, 182 patients with the severe closed craniocerebral injury were treated at the Neurosurgical Department and Intensive Care Unit. All patients had GCS scores ranging from 3-8 points. A total of 111 subjects were admitted with an isolated craniocerebral injury, and another 71 patients were admitted with both a closed craniocerebral injury and at least one more severe injury to other organs or systems.

The mean age of all patients was 49.82 years, with a standard deviation of 21.5 years. The smallest percentages were in the first and fourth decades of life. Men were injured four times more frequently than women (79% vs. 21%). There was a significant difference in age among the groups concerning the outcome (according to GCS total score) (t-test, $p<0.001$, Table 1). The difference was significant between groups 1 and 2 and between groups 1 and 5. The lack of difference between groups 1 and 3 as well as 1 and 4 was most likely due to the small sample (Table 1).

Table 1. Influence of the patient age on outcome

Group	1	2	3	4	5
Outcome	Well recovery	Average inability	Heavy inability	Prolonged vegetative condition	Death
N	46	30	10	2	94
The mean age	36.76±19.11	50.90±20.59	51.40±20.03	45.00±28.28	55.80±19.61

Statistically speaking, it has been estimated that circumstances of injury, the time spent from the moment of injury until admittance to the neurosurgical institution and the initial loss of consciousness do not influence the outcome. Instead, the outcome is influenced by dynamics in the state of consciousness, medical assistance and arrival at the neurosurgical institution, previous cranial fractures, breathing function upon admission, oxygen blood saturation, and brain stem reflex (reaction of pupils to the light and corneal reflexes). The GCS results were statistically important for predicting the outcome as well as the degree of cerebral tissue damage that was determined during the CT scan. The treatment outcome for craniocerebral injury (CCI) and polytrauma is described in Table 2.

Table 2. CCI and polytrauma and treatment outcome.

Outcome	CCI	Polytrauma	Total
Well recovery	32	14	46
Average inability	17	13	30
Severe inability	7	3	10
Vegetative condition	0	2	2
Death	55	39	94
Total	111	71	182

treatment outcome does not depend on injury type ($p = 0.252$, chi –square test)

A multifactorial linear regression showed that the craniocerebral injury outcome depended on age, oxygen saturation, GCS and severity of cerebral tissue damage, where the latter was determined during the CT scan (Table 3). The function representing the linear connection between the outcome and listed factors is shown in the formula: $i = 9.95 + 0.02 \times age - 0.66 \times SATO_2 - 0.36 \times GCS + 0.55 \times SCAN$, while $i = 1, 2, 3, 4, 5$ (according to the Glasgow Outcome Scale). Multivariate binary logistic regression showed that four separate factors influence the overall outcome: age, saturation by O_2, GCS and CT scan (Table 4).

Table 3. Variables influencing the treatment outcome.

Parameter	Coefficient	p
10 (Constant)	9.9470	0.000
AGE	0.0215	0.000
$SATO_2$	-0.0641	0.014
GCS	-0.3620	0.000
SCAN	0.5500	0.034

Table 4. Variables influencing the fatal outcome.

Parameter	p	Relative risk	Confidence interval
AGE	0.0030	1.0260	1.009 – 1.044
$SATO_2$	0.0230	0.8930	0.809 – 0.984
GCS	<0.0001	0.6430	0.520 – 0.795
SCAN	0.0240	2.5930	1.133 – 5.906

By employing the ROC and AUROC measurements, we found that patient's age (AUROC=0.667, p<0.001), SATO2 (AUROC=0.649, p=0.001), GCS (AUROC=0.740, p<0.001), CT scan findings (mild and severe injuries seen on CT) (AUROC=0.641, p=0.001) can be used as markers for the fatal outcome prediction (Figures 1 to 5). Using a linear model, we found that the combination of these variables made a better predictor of the fatal outcome (AUROC=0.813, p<0.001) compared to the individual variables alone. The cut-off for the model was 3.21, meaning that values were positive if higher than 3.21 and negative if lower. In 75% of the cases, we found positive results for the fatal outcome. Sensitivity (i.e., the relation of the number of those who did not survive and the number of those who survived) of this test was 79.79%. Specificity (i.e., the relation of the number of those who survived to the number of those with a negative test) of this test was 70.45% (Table 5).

Table 5. The test results (in groups, number of patients).

Test results	Dead	Survived
Positive test (> 3.21)	75	26
Negative test (≤ 3.21)	19	62

DISCUSSION

Statistics have shown that 37% of all injuries are closed craniocerebral injuries, and that 60% of patients with craniocerebral injuries have another injury. Polytrauma is of particular importance, as people with multiple traumas typically suffer an injury to the head. Studies have found that the mortality rate of patients with an isolated cranial injury was 11%. If the patient suffered from polytrauma, however, this increased to 21.8% (10). The most common craniocerebral injuries are caused by traffic accidents, falls, industrial injuries, sports injuries, as well as intentionally inflicted injuries (punches to the head, war injuries).

In Serbia, as well as in the former Serbia, Montenegro and Yugoslavia, complete data regarding the incidence of cranial injuries do not exist. The inconsistency in the data was influenced by the political climate over the last 15 years. In former Yugoslavia, 70 thousand people were injured annually, with a morbidity outcome of 4500-5000 people. Data from 1995 showed that 781 people died due to head injury, which is 7.4 per 100 thousand citizens. Furthermore, 5500 people, that is, 50 and 100 thousand citizens, were hospitalised in 1995, which means that the incidence of injury was 200 per 100,000 (10).

In addition to the symptoms of high intracranial pressure, the clinical definition of cranial injury is characterised by the focal symptoms that depend on the localization and size of the contusion or haemorrhage. An acute cerebral oedema with hyperventilation and the signs of decerebration is characteristic of younger age (8). This is because of blood congestion in the white cerebral matter during the disturbance in vasoregulation. The patients with the most severe craniocerebral injuries also have severe injuries to other organs and organ systems in various combinations. Severe injuries to other systems and organs are responsible for secondary cerebral damage.

The clinical picture can be different for different patients. A cranial injury can result in a deep coma, with dilated, atonic pupils, light insensitivity, silent corneal reflexes as well as other brain stem reflexes, atonic muscles and problems ranging from difficulties breathing to apnea. This condition is defined as an irreversible coma. In

the case that this condition lasts too long, it is followed by hypothermia, tachycardia, then hyperthermia, circulatory collapse and distress, ultimately leading to a lethal outcome. This condition can last for several hours to several days (10). After patients with slightly milder craniocerebral injuries come out of the coma, there is the risk of complications and aggravation during a period of at least three weeks (8). Monitoring of these patients is necessary so that the aggravation signs can be noticed on time. Symptoms such as an increase of the perifocal oedema, development of ischemic cerebral lesion, enlargement of some intracranial haematomas, aspiration pneumonia, acute respiratory distress syndrome (non-cardiogenic lung oedema due to the enlargement of permeable lung blood vessels) and haematemesis are most the common aggravation signs to look for.

The estimation of injury severity has not only significant, but also an outstanding prognostic value. Many factors influence the outcome that are not directly correlated with the injury, and are usually unavailable for research or unrecognisable at the time of the initial injury. Reported data and everyday practice have shown that injuries classified as mild can aggravate and have lethal consequences, contrary to the injuries that are, according to the defined criteria, classified as severe and can have a positive outcome. A typical example is an injured person with a brain concussion and cranial fracture. The patient's consciousness is inhibited and, during the development of an epidural haematoma, his condition deteriorates drastically such that immediate neurosurgical operation is necessary. The classification of injuries by severity is therefore of serious prognostic significance, but what is necessary here is warning before coming to a conclusion. It is clear that monitoring the patient is required so that all signs of aggravation are detected in a timely manner and the reaction can be adequate.

The outcome of severe craniocerebral injury is divided into five grades: good recovery, mild disability, severe disability, prolonged vegetative condition, and death. After completion of treatment, a patient with a severe craniocerebral injury can have long-lasting motor, cognitive, emotional and social disorders. The appropriate rehabilitation that enables these skills can be performed. The term "prolonged vegetative condition" is characterised by the inability to alternate between the sleeping and awake state. These patients are not communicative and they do not have any trace of mental activity. Severe instability means dependence on other persons. These patients are aware, communicative and able to help themselves. Moderate inability means that significant episodes may appear but the patients are able to help themselves and have a certain capability of professional rehabilitation. In patients that show good recovery, subsequent episodes can occur, but these episodes do not significantly influence their professional activity and these patients do not require help from others. The significant prognostic factors are age, coma duration and post-traumatic amnesia duration (11). The most intensive recovery period is during the first six months. There are some cases of unexpected recovery after a longer period, giving greater significance to the use of complex rehabilitation measures (10).

The most important prognostic factors for the treatment outcome are age, saturation with oxygen, GCS score and degree of the cerebral tissue damage visualised on the CT scan. When evaluating an age increase of one year, the mortality risk rises by 2.6% when the other parameters remain unchanged. If only oxygen saturation increases by 1%, the risk of the mortal outcome decreases by 10.7%. When evaluating a 1 point increase in the GCS, the mortality risks decrease by 36.7%, but only if the other parameters remain stable. A 159.3% increase in morbidity risk rate was observed between mild and severe brain injury when other parameters did not change. Finally, we found that the severe closed CCI is a dominant factor for the polytrauma outcome.

Our results are similar to previous findings in which two main prognostic factors were found: the admission status and extent of brain injury (16). To our knowledge, no study has investigated the oxygen saturation status in peripheral tissues following traumatic brain injury. It is well known that the most dangerous factor in brain injury is unresolved tissue hypoxia (17, 18). Neurological injury after brain trauma is directly correlated with severity of primary brain lesion (19). In our study, the mean age of all patients was 49.82 years, with a standard deviation of 21.5 years. The smallest percentages of the injured were in the first and fourth decades of life, which is consistent with previous findings. The percentage of cranial injuries increases at the age of 25. The risk then decreases and rises again after 50 years of age (20). Other studies have shown that the greatest number of injuries occurs up to 25 years of age (21) and that the average age of people suffering from isolated closed craniocerebral injuries was 28.6 ± 17.8 (22). Here, men were four times more at risk than women were (79% vs. 21%). We found that polytrauma occurred more often in accidental injuries (51.65% compared to 41.44%). Furthermore, polytrauma is often developed when falling down from a great height.

In conclusion, the results of our study show that age, $SATO_2$, GCS and degree of cerebral damage (defined by CT scan results) are predictors for a prognosis of the lethal outcome. The most accurate measure is made by the linear model that combines these variables. The cut-off for this marker is 3.21, meaning that the result is positive if it is higher than 3.21 and negative if it is lower. Finally, the test sensitivity was 79.79% while the specificity was 70.75%.

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UDK 616.42-006.04 / Ser J Exp Clin Res 2008; 9 (3): 111 – 115

BURKITT'S LYMPHOMA AS POSSIBLE CAUSE OF ACUTE ABDOMEN – CASE REPORT

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BURKITT-OV NON HODGKIN-OV LIMFOM KAO MOGUĆI UZROK AKUTNOG ABDOMENA – PRIKAZ SLUČAJA

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Received / Primljen: 24. 03. 2008. Accepted / Prihvaćen: 04. 08. 2008.

ABSTRACT

The term acute abdomen refers to acute abdominal conditions presenting with sudden, severe pain and signs of inflammation or dysfunction of abdominal organs, which in basics can be defined by variety of diseases.

A 19-year-old Caucasian male was admitted to the Emergency Department of the Clinical centre in Kragujevac with the condition of acute abdomen. The appropriate diagnostic procedures revealed a neoplastic process localised at the omentum majus, on the small stomach curve, in the hepatic hilus, small bowel mezo, sigmoid colon, vermiform appendix and associated lymphoid nodes, with diffuse infiltration of the small bowel wall, especially in the terminal part of jejunum. These clinical features are general characteristics of abdominal Burkitt's lymphoma and correspond to a sporadic form of Burkitt's lymphoma with nodal and extranodal localisation, negative for HIV infection. This form constitutes less than 1% of adult lymphomas worldwide.

In conclusion, there are various manifestations of Burkitt's lymphoma that have overlapping symptoms and signs with other intra-abdominal diseases. Although the presented case may not be a typical example of Burkitt's lymphoma, it is undoubtedly an aggressive lymphoma that requires an intensive and broad diagnostic approach and appropriate therapy, especially for the many intrinsic entities such as acute abdomen.

Key words: acute abdomen, Burkitt's lymphoma

SAŽETAK:

Termin akutni abdomen označava sva akutna stanja praćena jakim bolovima u stomaku i znacima inflamacije i/ili disfunkcije organa trbušne duplje koja se mogu javiti tokom različitih oboljenja.

Bolesnik star 19 godina primljen je u Urgentni centar Kliničkog centra Kragujevac zbog bolova u stomaku nejasne etiologije i otežanog disanja. Nakon sprovođenja neophodnih dijagnostičkih procedura, otkriven je neoplastični proces koji je bio lokalizovan na omentumu majusu, maloj želudačnoj krivini, u hilusu jetre, malom crevnom mezou, sigmoidnom kolonu, crvuljku kao i pridruženim limfnim čvorovima sa difuznom infiltracijom zida tankog creva, pretežno jejunuma. Posle učinjene eksplorativne hiruške intervencije i učinjene biopsije postavljena je i patohiostološka dijagnoza – Non Hodgkin limfom, tipa Burkitt. Klinička slika odgovara sporadičnoj formi Burkittovog limfoma sa nodalnom i ekstranodalnom lokalizacijom, HIV negativnog serotipa.

Ovaj klinički slučaj govori da se Burkittov limfom može ispoljiti simptomima i znacima karakterističnim za različite bolesti intraabdominalne lokalizacije. Iako prezentovan slučaj ne predstavlja tipičan primer Burkittovog limfoma, svakako nam ukazuje da ovako agresivan limfom zahteva intenzivan i širok dijagnostički pristup i odgovarajuću terapiju, pogotovo u takvim klinički intrigantnim entitetima kakav je akutni abdomen.

Ključne reči: akutni abdomen, Burkitt-ov limfom

INTRODUCTION

The term acute abdomen refers to acute abdominal conditions presenting with sudden, severe pain and signs of inflammation or dysfunction of abdominal organs1. However, it cannot be considered as a final diagnosis. It represents, in many cases, a condition requiring urgent management, even before all necessary diagnostic procedures are completed1,2. The patient described herein was admitted to the urgent medical care unit with the condition of acute abdomen, which was determined to be abdominal Burkitt's lymphoma.

Burkitt's lymphoma (BL) is a highly malignant, aggressive and rapidly growing B cell non-Hodgkin's lymphoma[2], which has a low long-term survival rate[4,5]. According to the WHO Classification, there are three clinical variants of Burkitt's lymphoma: endemic, sporadic and immunodeficiency-associated types[6,7]. The endemic variant of Burkitt's lymphoma is related to EBV or malaria infection and usually occurs in African children, 4-7 years old, involving the jawbone and other facial bones, as well as kidneys, ovaries, gastrointestinal tract, breasts and other extranodal sites[6,7]. Sporadic Burkitt's lymphoma is an extranodal disease associated with no specific geographic or climate area and accounts for about 40% of all lymphomas in children and only 1%-2% of those in adults[5]. Although almost

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any organ of the body may be involved, the most common localisation is intra-abdominal, affecting the intestinal tract, ovaries, kidneys, omentum and Waldeyer's ring, but very rarely lymph nodes of any localisation[5,8]. Immunodeficiency-associated Burkitt's lymphoma occurs mainly in patients infected with HIV, but also occurs in acquired and congenital immunodeficient patients[9,10]. The incidence of certain subtypes of Burkitt's lymphoma depends mostly on geographic area, and incidence of associated conditions. These observations emphasise the importance of precise disease definition for biological and epidemiological studies.

CASE REPORT

In December 2007, a 19-year-old Caucasian white male was referred to the Emergency Department of the Clinical centre in Kragujevac with dyspnea, abdominal swelling and upper abdominal pain. The patient confirmed that the above-mentioned symptoms had started spontaneously 4 days previously, first with the developing dyspnea, followed by the feeling of abdominal swelling and subsequent pain. Physical examination revealed axillar lymphadenopathy with lymph nodes of diameter around 10 mm, silent pulmonary sound on the both sides in the middle and lower lung fields and a tense abdominal wall (preventing spleen and liver palpation) with direct signs of peritoneal effusion.

Laboratory findings for haemoglobin levels and leukocyte and platelet counts were normal. Coagulation tests were within normal reference values. The erythrocyte sedimentation rate, fibrinogen level and C-reactive protein were normal, which confirmed non-infectious aetiology. The other important biochemical blood parameters were lactate dehydrogenase (LDH), 6603 U/L; acidum uricum, 1045 umol/L; and creatine kinase, 218 U/L. Liver function was also altered, which presented in the serum levels of total proteins, 53 g/L; aspartate-aminotransferase (SGOT), 145 U/L; and alanine-aminotransferase (SGPT), 45 U/L; but without an increase in bilirubin levels. The serum IgG and IgM levels were lower than normal, while circulating immune complexes, C3, C4, rheumatoid factor, as well as IgA level, were within normal reference values. The virology report confirmed the absence of HBsAg, anti-HCV Ab and antiHIV Ab. The patient's oxygen and carbon dioxide partial pressures (pCO2 and pO2) in the arterial blood showed hypoxia and hyperkapnia. The patient was put on oxygen support, which made the further diagnostic procedures less stressful.

The chest X-ray showed pleural effusion in the right lung to the level of the second rib. After performing pleural punction of 800 ml of clear liquid, the biochemical analyses confirmed pleural exudate with an extremely high level of lactate dehydrogenase (17,920 U/L), which suggested lymphoproliferative disease. A bone marrow biopsy was performed for confirmation.

In further procedures, an abdominal ultrasound scan and thoracic and abdominal CT confirmed the presence of pleural effusion, but of reduced volume, conceivable oesophageal perforation and diffuse infiltration of convolutions of the small bowel, together with mesenterium and omentum majus, infiltrating vermiform appendix and right perirenal area with discrete findings of peritoneal infiltration (Figures 1, 2, 3). The liver was slightly enlarged, the spleen was 111 mm in cranio-caudal diameter, and both were surrounded by peritoneal free liquid effusion (Figure 4).

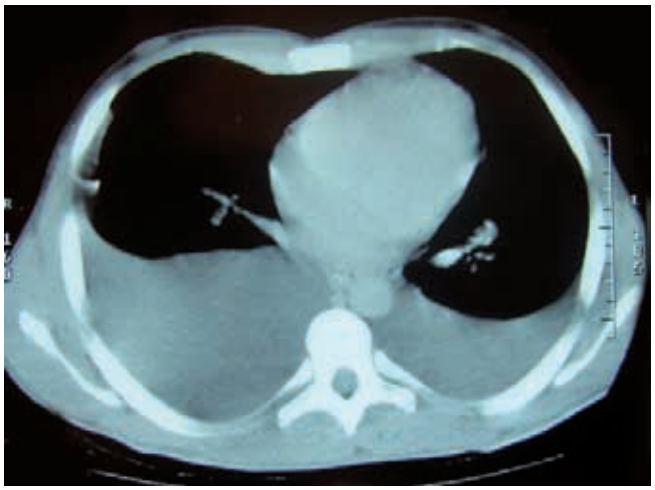

Figure 1. The presence of bilateral pleural effusion confirmed by the thoracic CT.

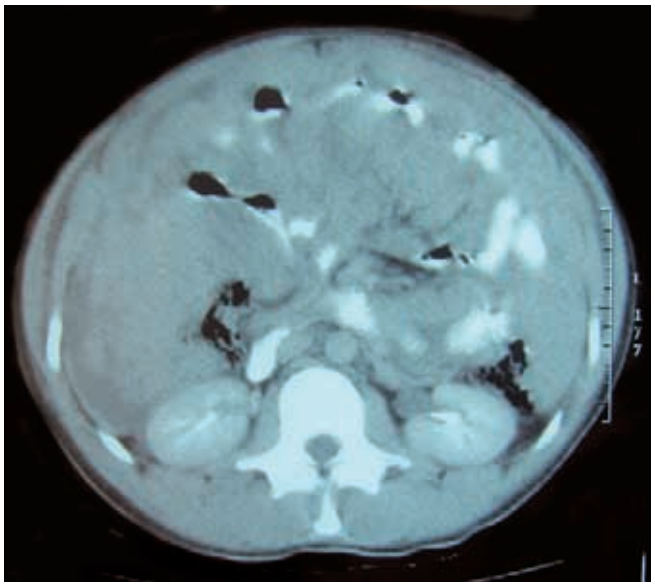

Figure 2. Diffuse infiltration of small bowel convolutions together with mesentery and greater omentum, infiltrating vermiform appendix and right perirenal area with discrete findings of peritoneal carcinosis.

The patient underwent explorative laparotomy, which confirmed the presence of neoplastic disease in the peritoneal cavity. Specifically, the disease was localised at the

omentum majus, on the small stomach curve, in the hepatic hilus, small bowel mezo, sigmoid colon, vermiform appendix and associated lymphoid nodes, with diffuse infiltration of the small bowel wall, especially on the terminal part of jejunum. *Ex tempore* biopsy confirmed malignant tissue and no other procedures were performed, except for the extirpation of an enlarged lymphoid gland of the small bowel's mezo for final histopathology diagnosis. Perforation of the oesophagus, or any other organ, was not confirmed.

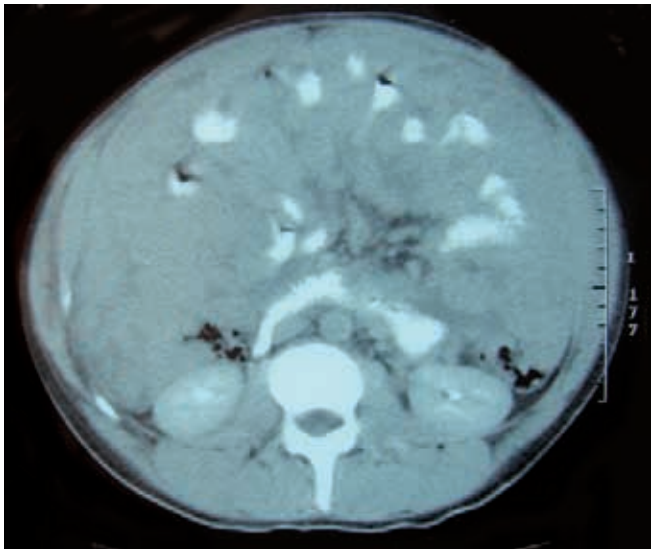

Figure 3. Diffuse infiltration of small bowel convolutions together with mesentery and greater omentum, infiltrating vermiform appendix and right perirenal area with discrete findings of peritoneal carcinosis.

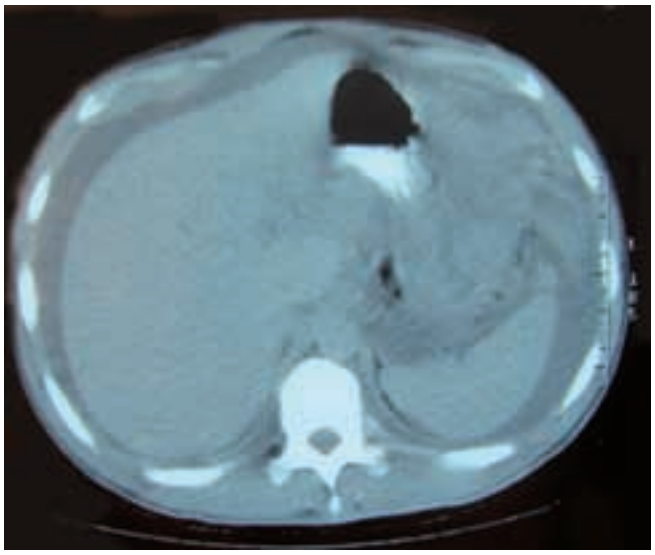

Figure 4. Normal size spleen and slightly enlarged liver were both surrounded with peritonal effusion.

On the histopathology report, the enlarged lymphoid node completely lost its anatomical structure and was infiltrated with the malignant cells, which gave it a "starry sky" appearance. Immunocytochemical stained cells were positive for CD79α, CD20 and Ki-67 (100%), and slightly positive for CD43 and CD10, which confirmed the diagnosis of high risk non-Hodgkin's lymphoma, B cell phenotype - Burkitt's lymphoma type (Figure 5). Bone marrow examination confirmed only non-specific reactive changes.

On completion of all the necessary diagnostic procedures, the patient's general condition deteriorated from day to day, resulting in renal, liver and pulmonary failure with a fatal outcome before any specific anti-neoplastic therapy could be applied.

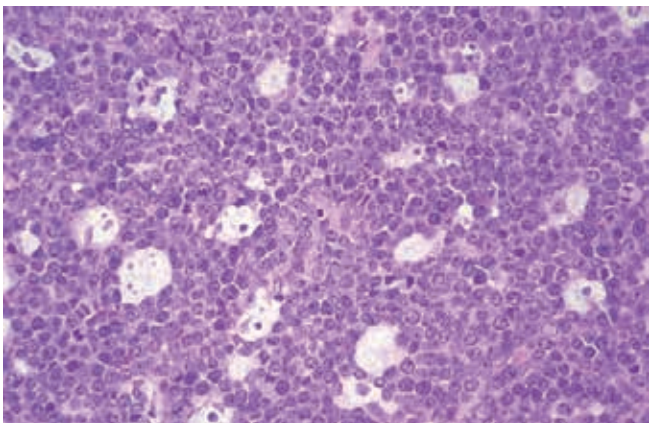

Figure 5. Light microscopy of the mesenterial lymphoid gland infiltrated with Burkitt's lymphoma cells, found in our patient (H/E, x40).

DISCUSSION

The diagnosis of acute abdomen continues to be one of medicine's most tempting tasks. Acute abdomen represents 5% to 10% of all emergency department visits[11]. The abdomen might be considered an incredibly intricate biological "black box", in which it is extremely difficult to pinpoint the source of distress. One of the main obstacles to the diagnostic process in patients with acute abdomen is the physician's own personal bias. A presumptive diagnosis is often reached before the data are fully collected, where such haste leads to overuse of tests and delays in establishing the correct diagnosis. Certain diseases develop an easily distinguished clinical picture in comparison to others, such as sickle cell crisis, mesenteric ischemia, intestinal obstruction and diabetes mellitus, which cause an onset of numerous clinical signs. The astute physician follows a progression of symptoms and signs of the disease over time and notes their onset, as well as their character and severity, in order to reach the correct final diagnosis.

As challenging as it is, careful history-taking, thorough evaluation of the symptoms, physical examination and a judicious use of laboratory tests can simplify the evaluation of the this acute condition. This article aims to illustrate one of the possible primary diseases loca-

lised to the abdominal cavity and not often considered as a cause of acute abdomen.

In 1958, Dennis Burkitt, a surgeon, first described a disorder presenting with jawbone tumours in African children[12]. He noted children with huge facial tumours uni- or bilaterally involving mostly jawbones, but also other facial bones, and sometimes accompanied by enormous abdominal masses. A few years later, the neoplasm was identified as a form of malignant lymphoma, and what initially emerged as a clinical syndrome became a pathological entity called Burkitt's lymphoma[13]. Histologically, Burkitt's lymphoma is composed of monomorphic, medium-sized neoplastic cells of lymphocyte origin with round nuclei, multiple nucleoli and relatively abundant basophilic cytoplasm. These cells typically possess an extremely high proliferation rate and high rate of programmed cell death (apoptosis). Morphological tumour characteristics are numerous, including admixed body macrophages phagocytosing abundant apoptotic debris, creating a typical "starry-sky" pattern[8]. The exact origin of the malignant cells is still unknown. Namely, the origin of these cells is currently thought to be a germinal centre B cell[14], although several studies on IgHV genes in Burkitt's lymphoma suggest that they may derive from memory B cells, rather than germinal centre B cells[15].

Burkitt's lymphoma is a unique tumour that sheds light on the understanding of lymphomagenesis. It is the first human neoplasm that is causally associated with viral infection (EBV). The c-*myc* gene that is expressed in Burkitt's lymphoma cells was the first oncogene to be described in lymphomas[16]. In addition, it is the first described non-Hodgkin's lymphoma associated with HIV infection. Among human neoplasms, Burkitt's lymphoma has the shortest doubling time.

There are three types of Burkitt's lymphoma. While the endemic or African types usually present with tumour masses localised on jawbone or retroperitoneum and strong association with Epstein-Barr viral infection, the sporadic type typically presents as an intra-abdominal tumour or digestive tract lesion[17], as seen in our patient. The sporadic type of Burkitt's lymphoma is often associated with HIV infection, especially in adults, and is rarely associated with Epstein-Barr infection[18]. On initial presentation, extranodal tumour localisation, predominantly in some intra-abdominal organs such as stomach, distal ileum, cecum or appendix, is most common[19,20,21]. Rarely, the disease is disseminated throughout almost all the intra-abdominal organs and lymph nodes, as described in this patient. Lymphoma cells were found in the omentum majus, on the small stomach curve, in the hepatic hilus, small bowel mezo, sigmoid colon and vermiform appendix, with the diffuse penetration in the small bowel wall, especially on the terminal part of jejunum. Because of this, the initial localisation of the disease could not be determined. Generally, the disease usually presents with mild and non-specific gastrointestinal symptoms and "B" symptoms, such as weight loss, unexplained fever and night sweats[22]. These symptoms are identical to those of numerous opportunistic infections and may delay the diagnosis of lymphoma. In the extreme, Burkitt's lymphoma can initially manifest with aggressive gastrointestinal symptoms and signs, which demonstrate an advanced disease requiring surgical treatment, as we noted in our patient[23]. Although the prognostic features have not yet been determined, some features that have been associated with adverse outcome in adults and children include older age, advanced disease, poor performance status, bulky disease, high level of LDH and involvement of central nervous system or bone marrow [24]. Almost all mentioned prognostic factors were present in our patient, culminating with a fatal outcome before any specific anti-neoplastic therapy could be applied.

Lessons learned: These findings indicate that there are different manifestations of Burkitt's lymphoma, which overlap with other intra-abdominal illnesses in symptoms and signs. Although the presented case may not be a typical example of Burkitt's lymphoma, it undoubtedly suggests an aggressive lymphoma that requires an intensive and broad diagnostic approach and appropriate therapy, especially in many intrinsic entities such as acute abdomen.

ABBREVATIONS

Ab – antibody
Ag – antigene
BL – Burkitt's lymphoma
C3,C4 – complement compartments
CRP – …… reactive protein
CT – computerised tomography
EBV – Epstein – Barr virus
HIV – Human immunodefficiency virus
IgA – Imunnoglobulin class A
IgG – Imunnoglobulin class G
IgM – Imunnoglobulin class M
LDH – Lactate dehydrogenase
pO_2 – Oxygen parcial pressure in arterial blood
pCO_2 – Carbon dioxide parcial pressure in arterial blood

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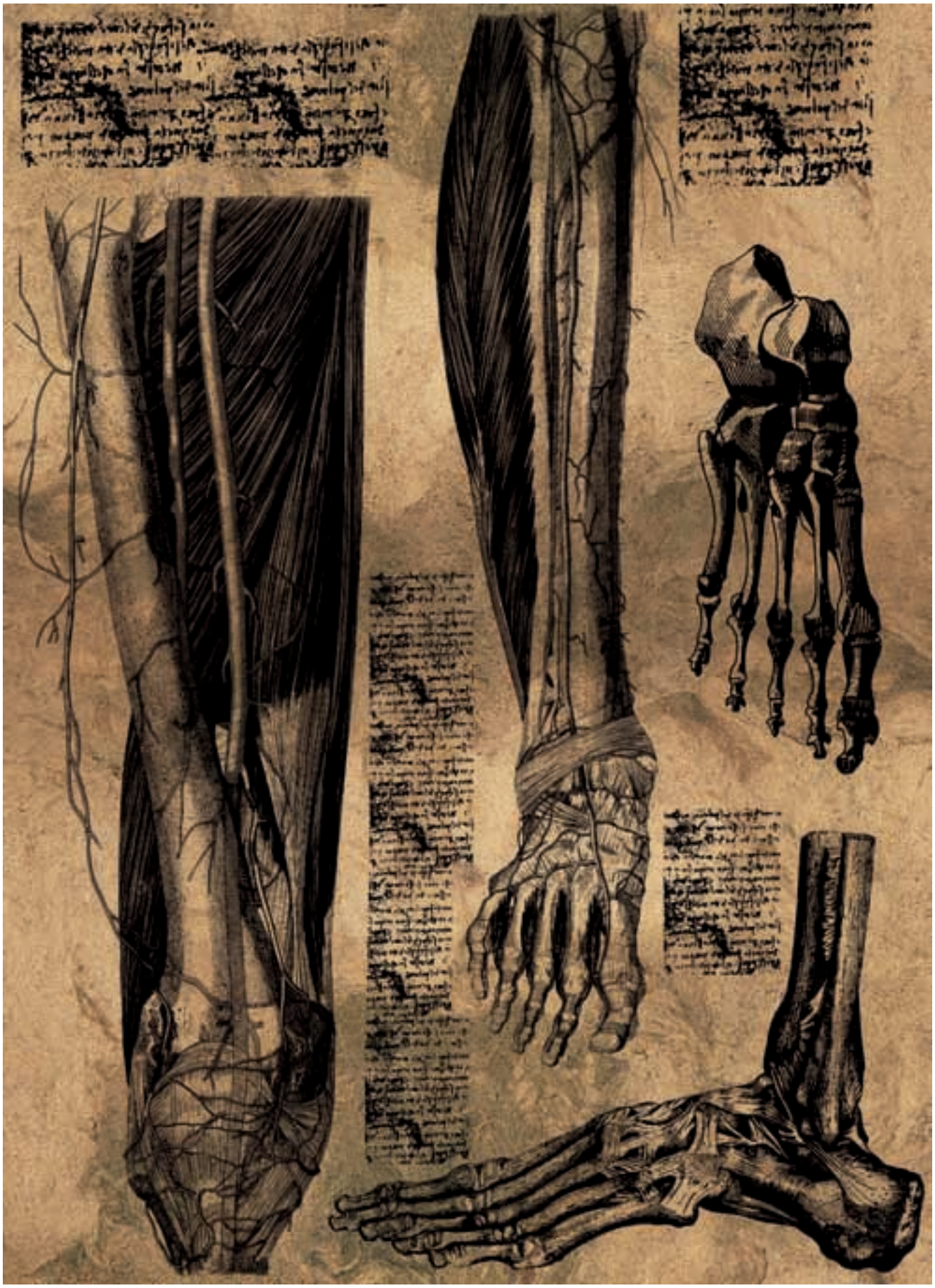

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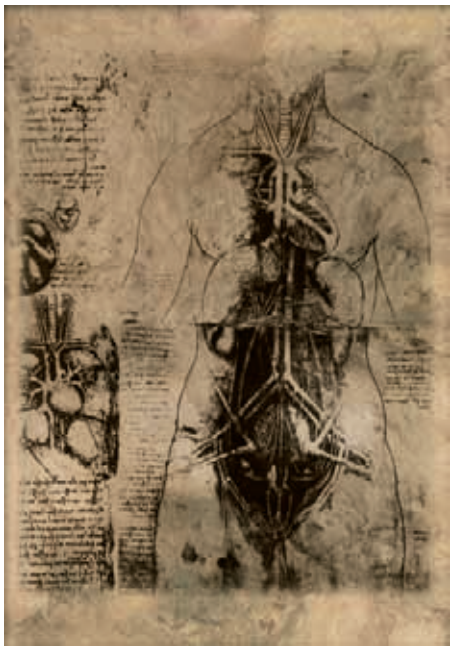

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Je nastavak: Medicus (Kragujevac) = ISSN 1450 – 7994
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