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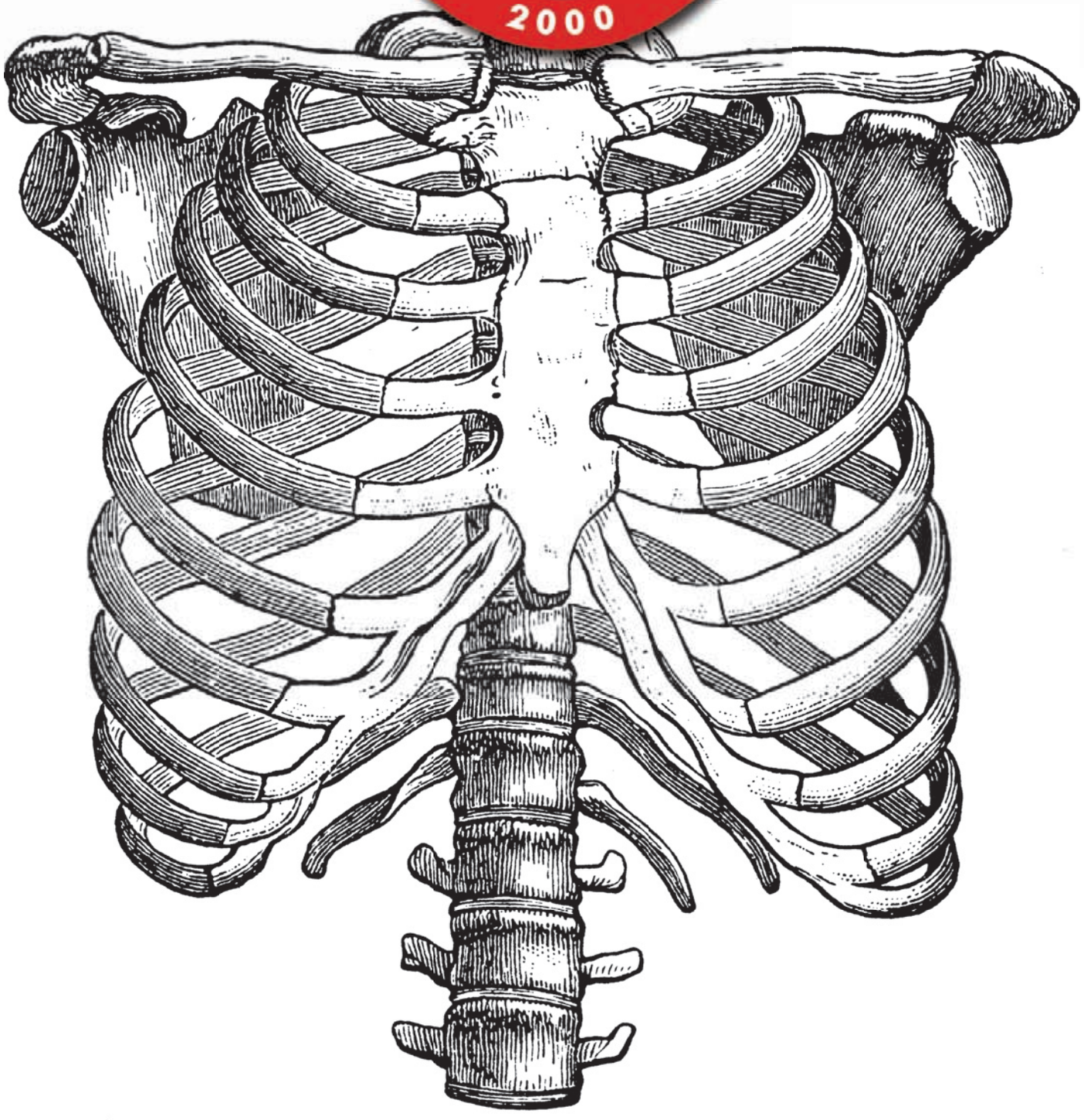

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A SHORT INTRODUCTION TO MARKERS OF STEM CELLS AND THEIR NEURAL DERIVATES

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Stem cells are primal cells common to all multi-cellular organisms that retain the ability to renew themselves through cell division (1). These cells can differentiate into a wide range of specialized cell types including cells of endoderm, mesoderm and ectoderm origin (2; Figure 1). The three broad categories of mammalian stem cells exist: embryonic stem cells, derived from preimplantation early embryos, adult stem cells, which are found in adult tissues, and cord blood stem cells, which are found in the umbilical cord. Embryonic stem cells (ESC) are pluripotent i.e. able to differentiate into all of the specialized embryonic tissues while adult stem cells and progenitor cells act as a repair system for the body, replenishing specialized and degenerated cells (3). Therefore, characterization of undifferentiated and differentiated stem cells is crucial for their application. In recent years, scientists have discovered a wide array of stem cells that have unique capabilities to self-renew, grow indefinitely, and differentiate or develop into multiple types of cells and tissues. Researchers now know that many different types of stem cells exist but they all are found in very small populations in the human body, in some cases one stem cell in 100,000 cells in circulating blood. So, how do scientists identify these rare types of cells found in many different cells and tissues-a process that is much similar to finding a needle in a haystack? The answer is rather simple thanks to specific stem cell markers. This review describes some of stem cell markers and their differentiation neural derivates.

I) EMBRYONIC STEM CELL MARKERS

Embryonic stem cells are stem cells derived from the early stage preimplantation embryo (3). Embryonic stem cells are pluripotent and able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes ESC from multipotent progenitor cells found in the adult since these only form a limited number of cell types. The presence of pluripotent adult stem cells remains a subject of scientific debate, however research has demonstrated that pluripotent stem cells can be directly generated from adult fibroblast cultures (4). The markers of most frequently used pluripotency markers are listed below. Alkaline phosphates (ALPL) also known as tissue-non specific isozyme precursor, is related to, but distinct from, intestinal/ALPI, placental/ALPP, and placental-like/ALPPL2 alkaline phosphates. An Alkaline phosphate is expressed at very high levels in undifferentiated human and mouse ESC, embryonic carcinoma (EC) cells, and embryonic germ (EG) cells (1-3). Interestingly, mutations in this enzyme have been linked directly to hypophosphatasia, a disorder that is characterized by hypercalcaemia and skeletal defects (5). Nanog is one of the most crucial pluripotency factors and this molecule is a member of the homeobox family of DNA binding transcription factors that has been shown to maintain pluripotency of ESC (6). Its expression is high in undifferentiated ESC and is down regulated during ESC differentiation, concomitant with loss of pluripotency (2). The second key factor of pluripotency is Oct-3/4. This is a 34 kDa POU transcription factor that is expressed in ESC and EG cells, and its expression is required to sustain cell self-renewal and pluripotency. Oct-3/4 is the most recognized marker for pluripotent ESC and reprogrammed (iPS) somatic cells (1, 2). Rex-1 is a zinc finger family transcription factor that is highly expressed in ESC (7) and one of several gene markers used to identify undifferentiated stem cells since its expression is down regulated upon stem

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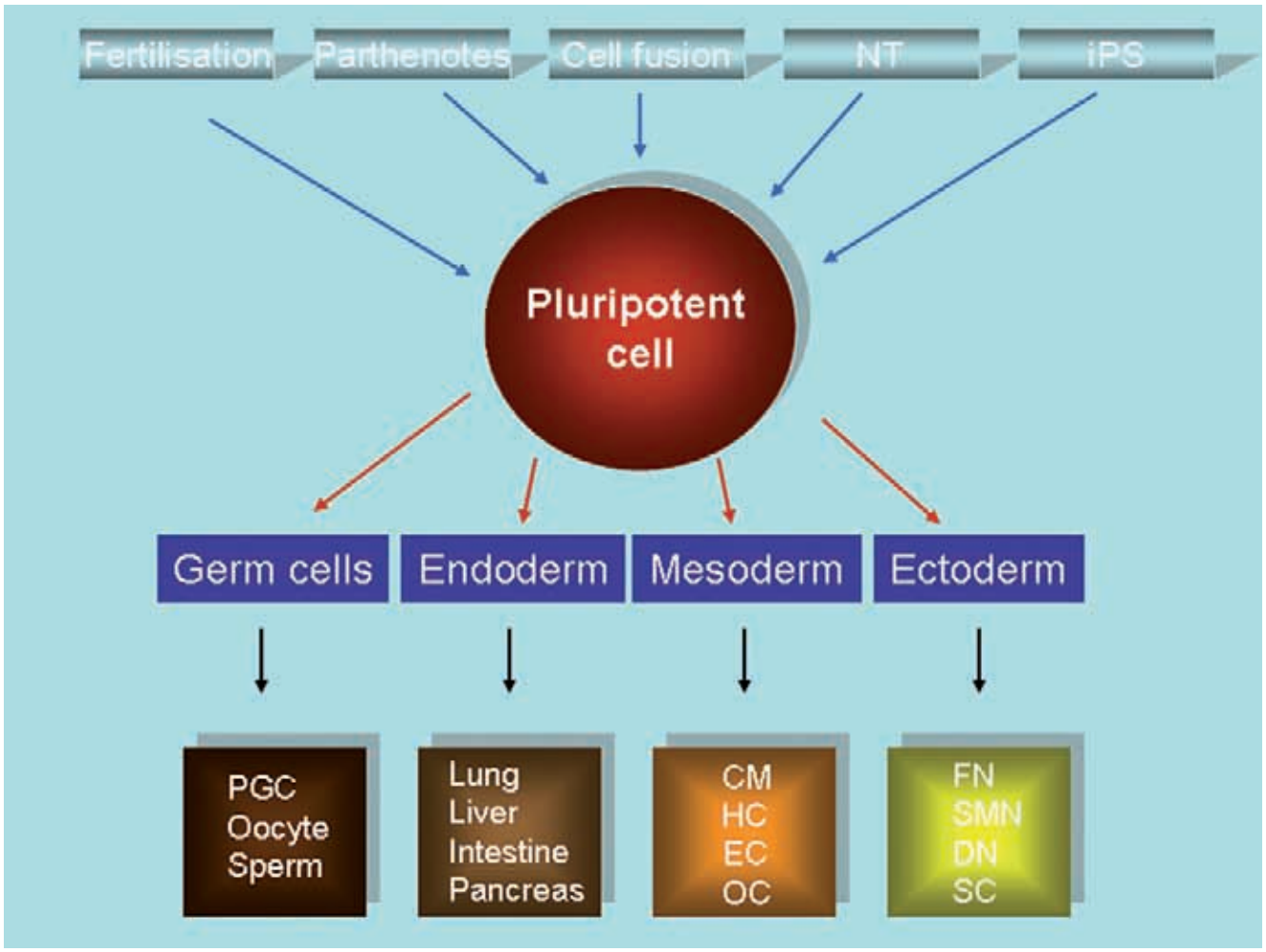

Figure 1: Pluripotent stem cells and their potential to differentiate. Abbreviations: NT: nuclear transfer; iPS: induced pluripotent stem cells; PGC: primordial germ cells; CM: cardiomyocytes; HC: hematopoietic cells; EC: endotheilial cells; OC: osteogenic cells; FN: forebrain neurons; SMN: spinal motor neurons; DN: dopaminergic neurons; SC: skin cells.

cell differentiation (2). Members of the large SOX family of transcription factors are widely conserved and at least 20 are found in mammals. Structurally SOX proteins exhibit a high mobility group motif that binds the DNA minor groove and these proteins play important roles in early development but these proteins are often used as markers to assess the differentiation of specific cell lineages (8). Stage specific embryonic antigens (SSEAs) were originally identified by monoclonal antibodies recognizing defined carbohydrate epitopes associated with lacto- and globo-series glycolipids (9). They are often used as markers for stem cell differentiation. SSEA-1 is expressed on murine EC cells, ESC, and primordial germ cells and SSEA-3 and SSEA-4 are synthesized during oogenesis and are present on oocyte, zygote, and early cleavage-stage embryo membranes (9). Murine SSEA-1 expression decreases with differentiation as SSEA-3 and SSEA-4 expression increase. In contrast, human EC and ESC express SSEA-3 and SSEA-4, and differentiation is accompanied by an up regulation of SSEA-1 and down-regulation of SSEA-3 and SSEA-4. The SSEA 1, 3 and 4 are globoseries glycolipids recognized by monoclonal antibodies originally raised to distinguish early stages of mouse development. Primate pluripotent cells express SSEA-3 and SSEA-4 (the epitope recognized by the latter is more readily detected than that seen by the former), and express SSEA-1 only upon differentiation (2, 9). Other cell-surface markers are TRA-1-60, GTCM-2 and TRA1-80. TRA1-60 epitope is a sialidase-sensitive epitope associated with this proteoglycan; the antibody GCTM-2 reacts with its core protein, and antibody TRA-1-80 reacts with other unknown epitopes on the same molecule (10). Human ESC, as well as monkey ESC reacts with TRA1-60, TRA1-80 and GCTM-2. Although GCTM-2 and TRA1-60 do not label mouse ESC or EC cells, it is not clear whether the mouse cells lack the surface proteoglycan or whether the antibodies are species specific.

II) HEMATOPOIETIC STEM CELL MARKERS

Hematopoietic stem cells (HSC) are stem cells found in bone marrow that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutro-

phils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells). However, the definition of HSC has undergone considerable revision in the last two decades since there are numerous evidences that these cells might be pluripotent in their primitive nature (11). In reference to phenotype, HSC are identified by their small size, lack of lineage (lin) markers, low staining with vital dyes such as rhodamine 123 or Hoechst 33342 and presence of various antigenic markers on their surface, many of which belong to the cluster of differentiation series. The most used markers are: CD34, CD38, CD45, CD90, CD133, CD105, and also c-kit-, the receptor for stem cell factor. CD34 remains the main marker of the HSC population but also a marker for multipotent stem cells present on lineage-committed hematopoietic progenitors from bone marrow and a subpopulation of immature thymocytes (12). CD133 (Prominin-1) is a HSC marker but also neural marker which is expressed in adult human differentiated cells and certain types of kidney cancer (13). Meanwhile CD1333 is a novel marker for human prostatic epithelial stem cells (14). During the purification of HSC by FACS method, a group of up to 14 different mature blood-lineage marker could be identified: CD13 and CD33 for myeloid, CD71 for erythroid, CD19 for B cells, CD61 for megakaryocytic (human) and CD45 for B cells, Mac-1 (CD11b/CD18) for monocytes, Gr-1 for Granulocytes, Ter119 for erythroid cells, Il7Ra, CD3, CD4, CD5, CD8 for T cells (mice). These antibodies are used as a mixture to deplete the lin+ cells or late multipotent progenitors (12-14).

III) NEURAL CELLS

Stem cells are extremely useful for furthering our understanding of both normal and abnormal human development, providing a human cell preparation that can be used to screen for new reagents and generating large numbers of differentiated cells that can be used for transplantation purposes. Critical among the applications for the latter are diseases and injuries of the nervous system, medical approaches to which have been, to date, primarily palliative in nature. Differentiation of human pluripotent stem cells into cells of the neural lineage therefore has become a central focus which has resulted in the description of several numerous methods for neural cell differentiation from human pluripotent stem cells (15, 16). Among these are methods for the generation of such divergent neural cells as dopaminergic neurons, retinal neurons, ventral motoneurons, and oligodendroglial progenitors (Figure 2). Some of the markers frequently used for identification of neural cells are listed below. While acetyl cholinesterase is a marker of early neuronal development (17), Musashi-1 is an evolutionally conserved marker for central nervous system (CNS) progenitor cells including neural stem cells (NCS; 18).

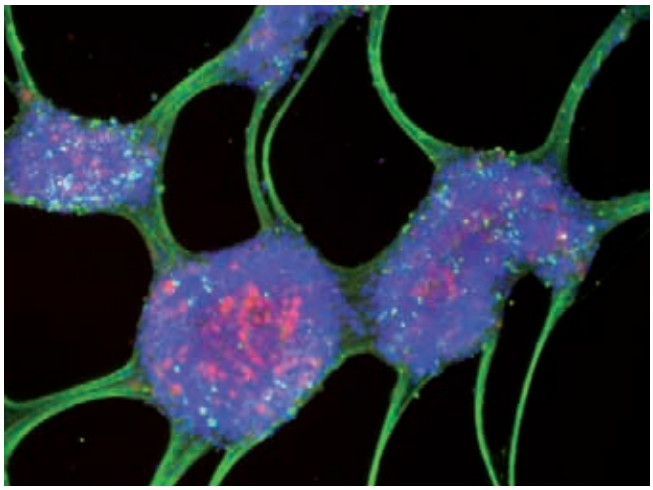

Figure 2: Differentiated human embryonic stem cells express TUJ-1 (green) and TH (tyrosine hydroxylase, marker for catecholamine-synthesising neurons; red) markers. Nuclei stained with DAPI (blue). Scale bar: 10 μm.

As a putative NCS marker Nestin is expressed in different areas of the adult mammalian brain that are known to support mitotic activity (19). Widely used marker of NCS is also Sox1 which is a transcription factor from the SoxB1 subgroup (20) while SOX2 is a persistent marker for multi potential NCS derived from ESC (21). A marker of axonal sprouting in mid stages of embryonic development is ELF which probably plays important role in NSC development (22). One of the earliest markers to signal neuronal commitment in primitive neuroepithelium is beta-tubulin (23). There are seven classes of beta-tubulin gene, one of which, class III (TUJ-1), is neuron specific (23) and was found in medulloepithelial rosettes. MAP2 is a marker of neuronal differentiation as a neuron-specific protein that stabilizes microtubules in the dendrites of post mitotic neurons (24). This marker is essential for development of early neuronal morphology and maintenance of adult neuronal morphology and appears early in neuronal maturation of the neocortex, particularly in the sub plate region (25). Additional markers of developing neocortex are Pax6, Tbr2 and Tbr1 which are sequentially expressed by radial glia, intermediate progenitor cells, and post mitotic neurons (26). A Neuronal nuclus (NeuN) is a 46/48-kD nuclear phosphoprotein antigen used widely in research and diagnostics to identify post mitotic neurons (27, 28). This is a neuron-specific protein which is present in most neuronal cell types of vertebrates and a marker of neuronal maturation in early human fetal nervous system (29). NeuN expression per se is a reliable marker of proliferate capacity but levels of NeuN expression may also be indicative of the physiological status of a post mitotic neuron (28). One of the most important neurotransmitters is the neurotransmitter acetylcholine which is synthesized by choline acetyl transferase (ChAT). Choline acetyltransferase is a cholinergic neuron-specific marker

(30) and since its expression in the nervous system is restricted to cholinergic neurons ChAT serves as a specific marker for these neurons. ChAT activity of brain cholinergic neurons can be increased by nerve growth factor under both conditions, in vitro and in vivo.

IV) MARKERS OF NEURONAL DISEASES AND DAMAGES

The improvement of the protocols for in vitro differentiation of stem cells has lead to the development of numerous markers for identification of diseased or damaged tissue/cells. Neuron specific enolase (NSE) a common marker for both endocrine cells and enteric nerves has been also used as a marker of in vitro neuronal damage (31) and N-cetylaspartate (NAA), a marker of cellular dysfunction, neuronal loss or damage has been used to study acute brain injury (32). Neuron-specific enolase CSF (CSF-NSE) could be used as a quantitative marker of ischemic damage or as a non-disease specific marker for the neuronal degeneration in dementia disorders providing useful adjuncts in the assessment of neuroprotective drugs in stroke (33). There are several human neural disorders which are evident by certain reduced activity of different proteins. For instance, ChAT activity is markedly reduced in the affected brain areas and TG-1 has been identified as a marker for neuronal nuclei in Alzheimer's disease (34). In addition, neuroendocrine-specific protein C (NSP-C) a marker of neuronal differentiation is reduced in brain of patients with Down syndrome and Alzheimer's disease. One of the frequently used markers of human brain tumors is calcineurin (35) while protein kinase C (PKC) is an early and sensitive marker of ischemia-induced progressive neuronal damage in gerbil hippocampus (36). Taken together, there are numerous cell markers and for sure we mentioned some of them. The list of the markers is useful especially where differentiated or damaged tissue/cells need to be identified. However, not all markers are specific (for instance see CD133). Additionally, Nestin may not be a suitable marker solely for the identification of neuronal cells but also to identify endocrine precursor cells in the pancreas (37). Therefore, additional analysis of cell type(s) should be demanded including specific intracellular markers and where possible the specific functionality of the undifferentiated/differentiated cells.

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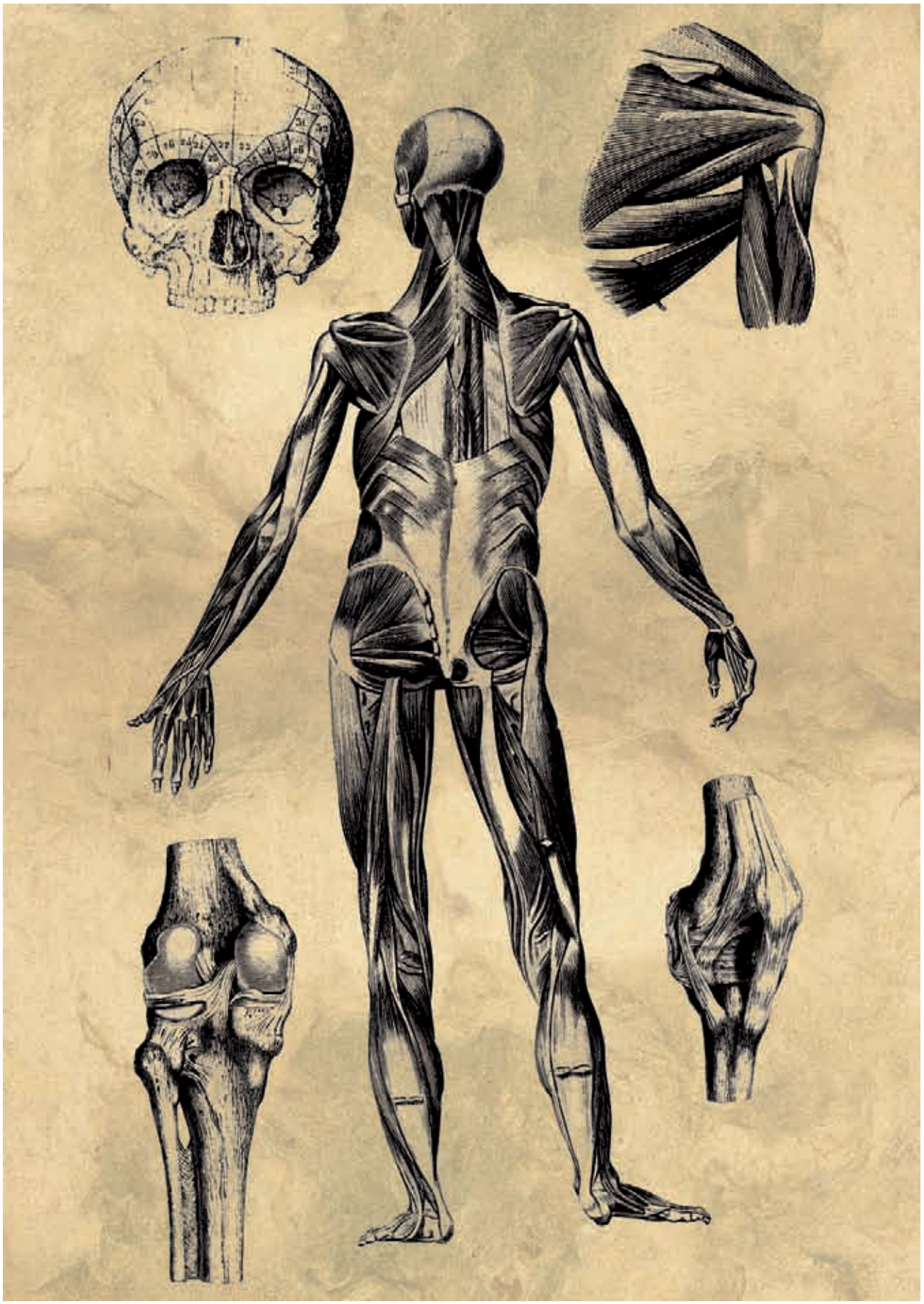

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LEFT VENTRICULAR HYPERTROPHY – RISK FACTOR FOR POOR OUTCOME IN HAEMODIALYSIS PATIENTS

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REMODELOVANJE LEVE KOMORE – FAKTOR RIZIKA ZA NEPOVOLJAN ISHOD BOLESNIKA KOJI SE LEČE REDOVNIM HEMODIJALIZAMA

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ABSTRACT

Background. Cardiovascular diseases are the leading cause of death in haemodialysis (HD) patients. Left ventricular hypertrophy (LVH) is a powerful predictor of cardiovascular morbidity and mortality in these patients. **Aim.** The aim of this study was to determine the prevalence of LVH, all cause and cardiovascular mortality, and to assess the predictive value of LVH for the outcome of HD patients during a two-year follow-up. **Methods.** The study included 115 patients (71 males and 44 females, average age 53.30 ± 12.17 years) on regular HD for the last 4.51 ± 4.01 years (average Kt/Vsp 1.17 ± 0.23). Patients were distributed in four groups according to LV morphology. **Results.** LVH was present in 82 (71.31%) patients. Patients with concentric LVH had significantly higher serum homocysteine then patients with normal LV morphology. Risk factors contributing to the development of LVH were anaemia, systolic hypertension, hyperhomocysteinaemia and low HDL-cholesterol. Anaemia is an independent risk factor for LVH in HD patients. The average two-year all-cause mortality rate in the examined patients was 13.74%. The mean two-year cardiovascular mortality rate was 8.51%. During a two-year follow-up period patients with an LV mass index (LVMi) >120g/m2 and end-diastolic volume index (iEDV) >90 mL/m2 had a significantly lower overall survival rate, while patients with LVMi >120g/m2 and iEDV ≤90mL/m2 had a significantly lower cardiovascular survival rate than patients with LVMi≤120g/m2 and iEDV≤90mL/m2. **Conclusion.** Left ventricle remodelling is a significant risk factor for poor outcome in patients on regular haemodialysis.

Key words: left ventricular hypertrophy, haemodialysis, mortality

SAŽETAK

Uvod. Kardiovaskularne bolesti su najčešći uzrok smrti bolesnika na hemodijalizi. Hipertrofija leve komore je snažan prediktor kardiovaskularnog morbiditeta i mortaliteta kod ovih bolesnika. **Cilj.** Cilj rada je bio da utvrdi prevalenciju hipertrofije leve komore, da utvrdi stopu opšteg i kardiovaskularnog mortaliteta, kao i da ispita prediktivnu vrednost hipertrofije leve komore za ishod bolesnika koji se leče hemodijalizom, u toku dvogodišnjeg praćenja. **Metod.** U radu je ispitano 115 bolesnika (71 muškarac i 44 žene) prosečne starosti 53,30 ± 12,17 godina, koji se leče redovnim hemodijalizama 4,51 ± 4,01 godina, prosečnog Kt/Vsp indeksa 1,17 ± 0,23. U zavisnosti od morfologije leve komore bolesnici su podeljeni u četiri grupe. **Rezultati.** Hipertrofiju leve komore ima 82 (71,31%) bolesnika koji se leče redovnim hemodijalizama. Bolesnici sa koncentričnom hipertrofijom imaju statistički značajno veću koncentraciju homocisteina u serumu u odnosu na bolesnike sa normalnom morfologijom leve komore. U faktore rizika koji doprinose razvoju hipertrofije leve komore spadaju anemija, povećan sistolni arterijski krvni pritisak, hiperhomocisteinemija i niska koncentracija HDL holesterola. Anemija je nezavisan faktor rizika za razvoj hipertrofije leve komore kod bolesnika koji se leče redovnim hemodijalizama. Prosečna dvogodišnja stopa opšteg mortaliteta ispitivanih bolesnika iznosi 13,74%, a prosečna dvogodišnja stopa kardiovaskularnog mortaliteta 8,51%. U toku dvogodišnjeg praćenja ispitivanih bolesnika, bolesnici sa LVMi>120 g/m2 i iEDV>90 ml/m2 imaju statistički značajno manju stopu preživljavanja od svih uzroka smrti, a bolesnici sa LVMi > 120 g/m2 i iEDV ≤ 90 ml/m2 imaju statistički značajno manju stopu preživljavanja od kardiovaskularnih uzroka smrti, u odnosu na bolesnike sa LVMi ≤ 120 g/m2 i iEDV ≤ 90 ml/m2. **Zaključak.** Remodelovanje leve komore je značajan faktor rizika za razvoj nepovoljnog ishoda bolesnika koji se leče redovnim hemodijalizama.

Ključne reči: hipertrofija leve komore, hemodijaliza, mortalitet

INTRODUCTION

Cardiovascular diseases are the leading cause of morbidity and mortality in patients treated with chronic haemodialysis (1-4). The annual cardiovascular mortality rate among these patients is 9%. The most frequent cardiovascular complications include left ventricular hypertrophy (LVH), ischaemic heart disease and congestive heart failure (1-4). Several risk factors, such as hypertension, anaemia, arteriovenous fistula, volume overload, oxidative stress, microinflammation, hyperhomocysteinaemia and disturbed calcium and phosphorus

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metabolism have been identified as stimulating LVH in the HD population (5).

Hypertension and atherosclerosis cause LV pressure overload which initiates remodelling: parallel addition of new sarcomeres and increased wall thickness at normal chamber radius (wall thickness/ventricle diameter ratio >0.45 - concentric LVH) (4-6).

Left ventricular volume overload caused by high water and salt intake, anaemia and increased arteriovenous fistula blood flow (Q_{AV}≥1000 mL/min) results primarily in the addition of new sarcomeres in series, and, secondarily, in the addition of sarcomeres in parallel. This results in increased LV wall thickness and LV diameter (h/r <0.45) - eccentric left ventricular hypertrophy (4-6).

Left ventricular hypertrophy evolves through two phases. The first is beneficial, adaptive hypertrophy as a response to the increased tensile stress of the LV wall. However, sustained volume and pressure overload lead progressively to a maladaptive hypertrophic response. This phase is characterized by the loss of myocardial cells, deterioration of systolic function and development of heart failure, eventually with lethal outcome (4-6).

The prevalence of LVH in patients on regular HD is 75%, making it an important predictor of cardiovascular morbidity and mortality in these patients (4, 5). The clinical strategy for decreasing all causes and cardiovascular mortality in HD patients includes early identification of high-risk patients, individual adaptation of the dialysis regime and maintaining a better haemodynamic and electrolyte balance (7-9). The strategy for identifying high-risk patients should include determining serum cardiac troponins (troponin I - cTnI and troponin T - cTnT), electrocardiographic parameters (QTc interval length and dispersion) and echocardiographic indices (LV mass index - LVMi and end-diastolic LV volume index - iEDV) (7-9).

Early detection of high-risk patients enables timely implementation of an adequate therapeutic approach. The primary therapeutic strategy for lowering the cardiovascular mortality rate in HD patients should include antiaggregation therapy, statins and beta-blockers, while the secondary strategy encompasses coronary revascularization and percutaneous implantation of a cardioverter defibrillator (PCD) (7-9).

The aims of this study were to determine the prevalence of risk factors for the development of LVH, to determine the independent risk factors for the development of LVH, to determine all cause and cardiovascular mortality, and to assess the influence of LV remodelling on outcome in HD patients.

PATIENTS AND METHODS

We studied 115 patients on chronic standard bicarbonate HD in the Haemodialysis Ward, Clinic for Urology and Nephrology, Clinical Center "Kragujevac" in Kragujevac. All patients were haemodynamically stable, virtually anuric (residual diuresis <200 mL/24h) and had been undergoing HD for at least 6 months. The follow-up period was two years. Patients presented neither clinical nor echocardiographic signs of acute coronary syndrome or congestive heart failure up to three months prior to the commencement of the study. All patients gave informed consent for participation in the study, according to the Declaration of Helsinki.

The following variables were analysed: haemoglobin, shunt blood flow (QAV), mean arterial blood pressure (MAP), serum albumin, homocysteine, C-reactive protein, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and lipoprotein (a), calcium, phosphate, intact parathyroid hormone (iPTH), cTnI and cTnt, and calcium-phosphorus product (Ca x PO4).

Laboratory studies

Blood sampling was performed after 12-hours of overnight fasting, before the HD session and heparin administration. Samples for the measurement of serum cTn were taken after 20 to 30 minutes of quiet resting in a semirecumbent position.

Haemoglobin concentration was determined by the colourimetric method (reference range 110-180 g/L). Haematocrit was determined automatically on a COULTER® A^C machine, based on the equation: Hct(%)= (RBCxMCV)/10 (reference range 0.35-0.60).

Serum albumin levels were measured by photometric colour test with bromcresol green. The normal range was 38 46 g/L and a concentration <36 g/L suggested malnutrition.

Serum calcium was determined with a photometric colour test (reference range 2.20-2.65 mmol/L) and serum phosphate with a photometric UV test (reference range 0.80-1.45 mmol/L). Serum iPTH was measured by radioimmunoassay (IRMA). The reference range for healthy persons is 11.8 - 64.5 pg/mL, while values between 200-300 pg/mL are considered appropriate for HD patients.

CRP serum concentration was determined using the immunochemical nephelometric method. It was calculated as the mean value from two measurements taken in three months. The normal concentration was ≤5 mg/L. Microinflammation was suggested when CRP was over 5 mg/L.

Total homocysteine serum concentration was measured by Fluorescence Polarisation Immunoassay (normal value ≤15 μmol/L).

Measurement of serum cTnT was performed based on electrochemiluminescence immunoassay technology (ECLIA method – ElektroChemiLumineszenz ImmunoAssay), using the Roche Diagnostics troponin T kit. The recommended diagnostic threshold for cardiac ischaemia is 0.1 ng/mL. Serum TnI was determined with ADV AxSYM cTnI immunoassay technology (Abbott laborato-

ries). A level of >0.15 ng/mL was considered positive for myocardial necrosis.

Echocardiographic study

All patients underwent echocardiographic examination on a SHIMADZU-2200 machine with a 2.5 MHz transducer probe. Examinations were performed 15 to 20 hours after the dialysis session by a single experienced cardiologist without previous knowledge of the subjects' clinical characteristics, in order to avoid end-diastolic LV diameter alterations induced by the interdialytic volume gain and subjectivity.

Left ventricular mass index, which is normally ≤131 g/m2 in men and ≤100 g/m2 in women, was used as an indicator of LVH. LVMi was calculated as follows:

$$\text{LVMi} = \frac{0.00083x\left[(LVEDD + IVSd + LVPWd)^3 - (LVEDD)^3\right] + 0.6}{BSA}\ \text{g/m2.}$$

The left ventricular end-diastolic volume index was quantified as follows:

$$\text{iEDV} = \frac{(LVEDD)^3 x 0.001047}{BSA}\ \text{mL/m2.}$$

Normally, iEDV is ≤90 mL/m2.

Left ventricle fractional shortening (LVFS), representing a measure of systolic function, is normally 42 ± 8%. It was calculated as follows:

$$\text{LVFS} = \frac{(LVEDD - LVESD)}{LVEDD} x100\ \%.$$

The left ventricular ejection fraction (LVEF) was calculated as an indicator of systolic function, based on the following equation:

$$\text{LVEF} = \frac{(LVEDV - LVESV)}{LVEDV} x100\% \ .$$

The normal range is 67 ± 9%.

Abbreviations in the formulas stand for: IVSd - interventricular septal wall thickness in diastole (mm), LPWd - LV posterior wall thickness in diastole (mm), LVEDD - LV end-diastolic diameter (mm), LVESV - LV end-systolic volume (mL), LVEDV - LV end-diastolic volume (mL), BSA - body surface area (m2).

Left ventricular hypertrophy was defined as IVSd >11 mm, LVPWd >11 mm and LVMi >131 g/m2 for men and >100 g/m2 for women (10-12). LV concentric hypertrophy was defined as IVSd >11mm, LVEDD >11mm, LVEDD <4.7 mm and LVMi >131 g/m2 in males and >100 g/m2 in females, with normal fractional shortening and relative LV wall thickness >45% (10-12). Eccentric LV hypertrophy is present when LVMi >131 g/m2 in males and >100 g/m2 in females, LVEDD >57 mm, with normal fractional shortening and relative LV wall thickness ≤45% (10-12). LV dilatation was defined as LVEDD >57 mm and iEDV >90 mL/m2, with normal systolic function and LVMi (10-12). Impaired systolic function was defined as LVFS ≤25% and LVEF ≤50% (10-12).

Arterial blood pressure (pre-dialysis pressure) was calculated as the average value of 12 measurements (3/week) taken during the month preceeding the study. Mean arterial pressure was calculated as diastolic blood pressure + 1/3x(systolic blood pressure minus diastolic blood pressure).

Shunt blood flow (QAV) was determined with colour-flow Doppler ultrasound just before echocardiographic examination, on a SHIMADZU-2200 machine, using the 7.5 MHz probe. Blood flow was calculated as the average value of three measurements on an efferent vein, 2-4 cm proximally to anastamosis. Adequate dialysis requires blood flow of 300 to 800 mL/min.

Haemodialysis adequacy was assessed by the Kt/Vsp index, calculated based on Daugridas' second-generation formula:

$$Kt/Vsp = -\ln(C_2/C_1 - 0.008 \times T) + (4 - 3.5 \times C_2/C_1) \times UF/W,$$

where C_1 stands for predialysis serum urea (mmol/L), C_2 - postdialysis serum urea (mmol/L), T - treatment time (h), UF - ultrafiltration (L) and W - body weight after dialysis (kg). Serum urea was determined with a complete enzymatic method (urease-glutamate-dehydrogenase), with the reference range being 3.5 - 7.5 mmol/L. According to K/DOQI guidelines, the Kt/V delivered should be ≥1.2.

Causes of death in the examined HD patients were defined as cardiovascular events (acute myocardial infarction, congestive hart failure and sudden cardiac death) and non-cardiovascular events (infection/sepsis, neoplasm, unknown) (7).

Statistical analysis

Data are expressed as mean ± SD. Results were statistically analysed with the t test, Mann-Whitney U test, one-factorial ANOVA, Kruskal-Wallis test, Bonferroni test, univariate and multivariate logistic regression analysis, Kaplan-Meier and Log-Rank tests for survival analysis. Values <0.05 and <0.01 were considered significant.

RESULTS

General patients' data are shown in Table 1. LVH was present in 82 (71.31%) patients on regular HD. Concentric LVH was present in 33 patients (28.70%) and eccentric hypertrophy in 49 (42.61%) patients. LV dilatation was found in 16 (13.91%) patients. Seventeen patients (14.78%) had normal echocardiographic finding (Table 1).

Hyperhomocysteinaemia as a risk factor for LVH was present in 86.09% of all patients, anaemia in 76.52%, hypertension in 36.52% and microinflammation in 34.78%. Increased shunt flow (QAV >1000 mL/min) had the lowest prevalence of all LVH risk factors (9.57%), as shown in Figure 1.

Table 1. Demographic and clinical data of patients classified in relationship to left ventricular (LV) remodelling status (echocardiographic assessment)

GENERAL DATA	PATIENT GROUP - LV MORPHOLOGY			
	Concentric LVH	Eccentric LVH	LV dilatation	Normal LV
	Mean± SD	Mean ± SD	Mean ± SD	Mean ± SD
Number (N/%)	33 (28.70%)	49 (42.61%)	16 (13.91%)	17 (14.78%)
Sex (male/female)	16/17	28/21	13/3	14/3
Age (years)	52.55 ± 12.40	54.02 ± 12.75	54.00 ± 7.72	52.29 ± 13.17
BMI (kg/m^2)	22.46 ± 3.54	22.13 ± 2.86	23.69 ± 2.94	22.60 ± 2.77
Time od dialysis (years)	3.82 ± 3.62	4.71 ± 4.13	4.31 ± 4.80	4.94 ± 3.94
Kt/Vsp index	1.28 ± 0.27*	1.10 ± 0.21	1.11 ± 0.17	1.19 ± 0.23
Systolic BP (mmHg)	140.76 ± 19.69	142.04 ± 21.41	130.63 ± 13.40	132.35 ± 22.23
Diastolic BP (mmHg)	86.06 ± 13.39	86.53 ± 13.32	80.00 ± 6.32	79.41 ± 11.44
MAP (mmHg)	104.29 ± 14.65	105.03 ± 15.65	96.88 ± 7.84	97.06 ± 14.81
Interdilytic weight gain-IDWG (%)	2.64 ± 0.81	2.52 ± 0.86	2.78 ± 0.82	2.79 ± 0.79
Total proteins (g/L)	73.91 ± 6.23	73.86 ± 5.13	73.44 ± 6.77	72.47 ± 8.48
Serum albumin (g/L)	39.73 ± 4.71	40.92 ± 4.06	40.69 ± 5.46	40.53 ± 5.04
C-reactive protein (mg/L)	6.98 ± 10.01	8.99 ± 10.40	7.74 ± 8.22	4.05 ± 3.74
Homocysteine (µmol/L)	25.11 ± 8.17**	23.94 ± 10.16	20.77 ± 6.47	18.82 ± 4.58
Troponin I (ng/ml)	0.20 ± 0.46	0.20 ± 0.51	0.28 ± 0.69	0.07 ± 0.16
Troponin T (ng/ml)	0.13 ± 0.16***	0.20 ± 0.32****	0.07 ± 0.09	0.03 ± 0.03
PRIMARY RENAL DISEASE				
Nephropathia diabetica	7 (21.21%)	4 (8.16%)	0 (0.00%)	0 (0.00%)
GN chronica	9 (27.27%)	16 (32.65%)	4 (25.00%)	5 (29.41%)
Nephropathia obstructiva	0 (0.00%)	3 (6.12%)	0 (0.00%)	1 (5.88%)
Nephropathia hypertensiva	7 (21.21%)	9 (18.37%)	3 (18.75%)	2 (11.76%)
Renes polycystici-RPC	5 (15.15%)	5 (10.20%)	1 (6.25%)	4 (23.53%)
PN chronica bilateralis	4 (12.12%)	10 (20.41%)	3 (18.75%)	3 (6.12%)
Nephropathia endemica	0 (0.00%)	1 (2.04%)	0 (0.00%)	1 (5.88%)
Nephropathia chronica	1 (3.03%)	1 (2.04%)	5 (31.25%)	1 (5.88%)

*PI, II <0.05, **PI,IV <0.01,***PI,IV <0.05, ****PII,IV <0.01
ANOVA, Kraskal-Wolis-ov test

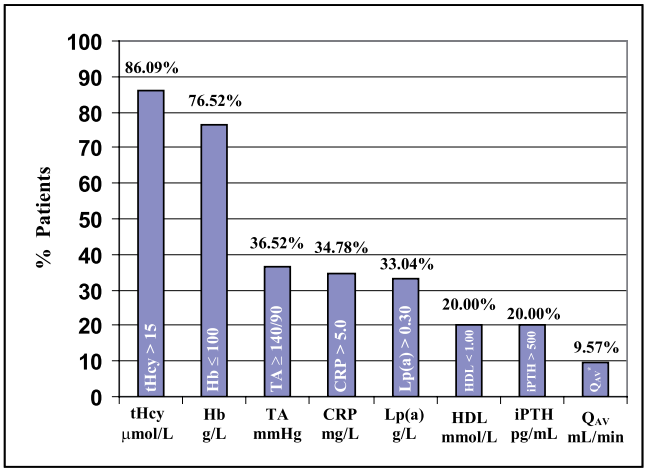

Figure 1. Prevalence of risk factors for left ventricular hypertrophy in haemodialysis patients

Patients with concentric LVH had a significantly higher dialysis adequacy index than patients with eccentric LVH ($p<0.05$), and significantly higher serum homocysteine ($p<0.01$) and cTnT ($p<0.05$) than patients with normal LV (Table 1).

Serum cTnT concentration was significantly higher ($p<0.01$) in patients with eccentric LVH than in those with normal LV mass (Table 1).

Univariate logistic regression analysis showed a significant negative correlation between HDL-cholesterol, haemoglobin, haematocrit and LVMi ($p<0.05$) (Table 2). A statistically significant correlation was found between arterial blood pressure, mean arterial blood pressure and LVMi ($p<0.05$) (Table 2).

Table 2. Univariate logistic regression analysis of risk factors for left ventricular hypertrophy

Variable	Logistic regression analysis						
	B	S.E.	R	Exp B	95%CI for Exp B		Significance (p)
					Lower	Upper	
Serum albumin	0.007	0.046	0.000	1.007	0.921	1.100	0.885
Cholesterol	-0.053	0.189	0.000	0.949	0.665	1.375	0.781
LDL cholesterol	-0.216	0.261	0.000	0.805	0.483	1.344	0.407
HDL cholesterol	-1.649	0.839	-0.117	0.192	0.037	0.995	0.049
Triclicerids	0.294	0.182	0.067	1.341	0.940	1.915	0.106
Hemoglobin	0.036	0.015	-0.161	1.036	1.006	1.067	0.019
Hematocryte	0.102	0.050	-0.128	1.107	1.005	1.220	0.040
C-reactive protein	-0.011	0.024	0.000	0.990	0.945	1.036	0.656
Homocystein	-0.039	0.028	0.962	0.962	0.911	1.015	0.160
Calcium-Ca^{2+}	0.588	0.841	0.000	1.801	0.346	9.361	0.484
Phosphate-PO_4^{3-}	0.038	0.409	0.000	1.039	0.466	2.317	0.926
Ca^{2+} x PO_4^{3-}	0.076	0.162	0.000	1.079	0.785	1.483	0.639
Parathormone-iPTH	0.000	0.001	0.000	1.000	0.999	1.001	0.888
Time on dialysis	0.004	0.052	0.000	1.004	0.907	1.112	0.934
Systolic blood pressure	-0.021	0.010	0.117	0.980	0.960	1.000	0.049
Diastolic blood pressure	-0.033	0.017	0.112	0.967	0.935	1.001	0.054
Mean arterial pressure	-0.029	0.015	0.122	0.971	0.944	0.999	0.045
Shunt flow-Q_{AV}	0.000	0.001	0.000	1.000	0.999	1.002	0.693
Interdilytic weight gain	0.334	0.253	0.000	1.396	0.851	2.291	0.187
Kt/Vsp index	-0.798	0.913	0.000	0.450	0.075	2.694	0.382

Multivariate logistic regression analysis showed anaemia to be an independent risk factor for LVH development (Table 3).

Table 3. Multivariate logistic regression analysis of risk factors for left ventricular hypertrophy

Variable	Logistic regression analysis						
	B	S.E.	R	Exp B	95%CI for Exp B		Significance (p)
					Lower	Upper	
HDL cholesterol	-1.486	0.867	-0.117	0.226	0.041	1.238	0.087
Hemoglobin	- 0.279	0.113	-0.083	1.322	1.059	1.650	0.014
Hematocryte	-0.843	0.373	-0.132	0.431	0.207	0.894	0.024
Systolic blood pressure	0.021	0.040	0.000	1.021	0.944	1.105	0.604
Mean arterial pressure	-0.057	0.056	0.000	0.945	0.847	1.055	0.311

HD patients with poor outcome had significantly lower serum albumin ($p<0.01$), higher serum homocysteine ($p<0.05$) and higher LVMi, cTnT and cTnI ($p<0.01$), than patients with good outcome (Table 4).

The average two-year all-cause mortality rate in our study group was 13.74%. The average two-year cardiovascular mortality rate was 8.51%.

During the two-year follow-up period, patients with LVMi >120 g/m2 and iEDV >90 mL/m2 had a significantly higher all-cause mortality rate then patients with LVMi ≤120 g/m2 and iEDV ≤90 mL/m2 (Log-Rank 4.72,

p <0.05) (Figure 2). Patients with LVMi >120 g/m2 and iEDV ≤90 mL/m2 had a significantly higher cardiovascular mortality rate then patients with LVMi≤120 g/m2 and iEDV ≤90 mL/m2 in the same period of time (Log Rank 4.07, p <0.05) (Figure 3).

Table 4. Demographic, clinical and echocardiographic data in relationship to outcome in the two-year study period

Variable	OUTCOME		Significance (p)
	ALIVE (86)	DECEASED (29)	
	Mean ± SD	Mean ± SD	
Sex (male/female)	56/30	15/14	χ^2_{emp}=1.647, p=0.199
Age (years)	52.47 ± 12.04	55.76 ± 12.41	t_{emp}=-1.264, p=0.209
Body mass index (kg/m^2)	22.76 ± 3.20	22.13 ± 3.15	t_{emp}=0.925, p=0.357
Time on dialysis (years)	4.50 ± 4.20	4.55 ± 3.45	Z_{emp}=-0.612, p=0.541
Dialysis adequacy-Kt/Vsp	1.18 ± 0.21	1.12 ± 0.29	t_{emp}=1.206, p=0.230
Systolic blood pressure (mmHg)	137.67 ± 20.39	139.48 ± 22.85	t_{emp}=-0.401, p=0.690
Diastolic blood pressure (mmHg)	83.95 ± 12.54	84.48 ± 14.10	t_{emp}=-0.190, p=0.849
Mean arterial pressure (mmHg)	101.86 ± 14.77	102.82 ± 16.19	t_{emp}=-0.294, p=0.769
Hemoglobin (g/L)	90.60 ± 14.74	86.90 ± 11.82	t_{emp}=1.227, p=0.222
Hematocryte (%)	26.89 ± 4.44	25.98 ± 3.51	t_{emp}=0.997, p=0.321
Total proteins (g/L)	73.38 ± 6.10	73.41 ± 6.49	t_{emp}=0.274, p=0.784
Serum albumin (g/L)	41.31 ± 4.36	38.31 ± 4.71	t_{emp}=3.145, p=0.002
Total cholesterol (mmol/L)	4.65 ± 1.11	4.50 ± 1.17	t_{emp}=0.653, p=0.515
LDL-cholesterol (mmol/L)	2.64 ± 0.82	2.56 ± 0.86	t_{emp}=0.422, p=0.674
HDL-cholesterol (mmol/L)	1.05 ± 0.28	1.07 ± 0.33	t_{emp}=-0.284, p=0.777
Triglycerids (mmol/L)	2.00 ± 1.18	1.78 ± 0.83	t_{emp}=0.968, p=0.335
Lipoprotein (a) (g/L)	0.26 ± 0.26	0.28 ± 0.26	Z_{emp}=-0.309, p=0.757
C-reactive protein (mg/L)	5.17 ± 5.64	14.39 ± 13.89	Z_{emp}=-1.150, p=0.250
Homocysteine (µmol/L)	22.25 ± 8.86	26.80 ± 8.42	t_{emp}=-2.419, p=0.017
Troponin T (ng/mL)	0.09 ± 0.12	0.30 ± 0.37	Z_{emp}=-2.476, p=0.013
Troponin I (ng/mL)	0.14 ± 0.46	0.35 ± 0.55	Z_{emp}=-4.893, p=0.0001
Left ventricular mass index (g/m^2)	138.02 ± 34.57	161.13 ± 53.61	t_{emp}=-2.681, p=0.008
End-diastolic volume index (mL/m^2)	98.46 ± 33.61	107.76 ± 37.18	t_{emp}=-1.254, p=0.212
Left ventricular fractional shoretning (%)	33.01 ± 7.35	31.04 ± 8.70	t_{emp}=1.191, p=0.236
Left ventricular ejection fraction (%)	68.86 ± 10.44	65.70 ± 12.79	t_{emp}=1.330, p=0.186

Chi-Square tests, Mann-Whitney U test, Student-ov T test

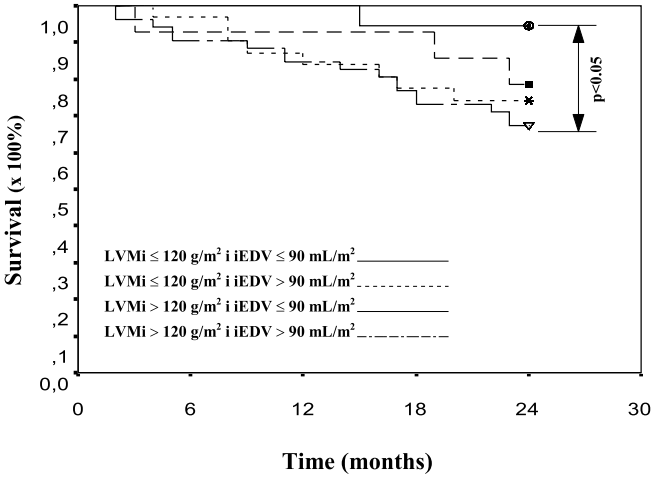

Figure 2. Survival rate from all causes of death in haemodialysis patients in relationship to left ventricular mass index (LVMi) and end-diastolic left ventricular volume (iEDV) during the two-year follow-up period

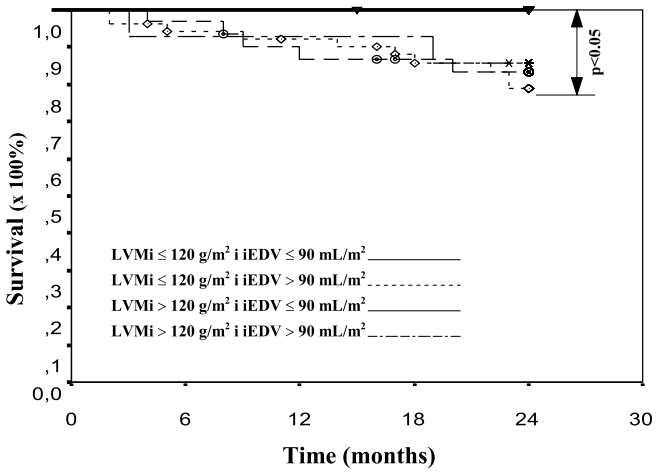

Figure 3. Survival rate from cardiovascular causes of death in haemodialysis patients in relationship to left ventricular mass index (LVMi) and end-diastolic left ventricular volume (iEDV) during the two-year follow-up period

DISCUSSION

The risk of cardiovascular complications in patients with end-stage renal disease (ESRD) is by far greater than in the general population (13, 14). An increased incidence of cardiovascular disease in HD patients is correlated with a high prevalence of traditional (hypertension, disturbed lipid metabolism, diabetes, smoking) and non-traditional (microinflammation, oxidative stress, hyperhomocysteinaemia, secondary hyperparathyroidism) risk factors, which lead to increased atherosclerosis, characteristic of ESRD patients, plaque destabilization, myocardial fibrosis and valvular heart disease (15-17). The average two-year cardiovascular mortality rate in our study group was 8.51%. Similar results were reported by other authors who found a one-year mortality rate of 9% (1).

Left ventricular hypertrophy was present in 71.31% of patients on regular HD. A similar rate was reported by other authors (18-21). The prevalence of LVH in chronic renal failure patients is approximately 40%, reaching 75% in ESRD (11). Timely identification of risk factors and adequate treatment results in LVH regression in HD patients (22).

Anaemia is present in over 90% of HD patients and represents an important risk factor for LVH. Haemoglobin ≤100 g/L was present in 76.52% of our patients. Multivariate logistic regression analysis identified anaemia as an independent risk factor for LVH in our study group. Similar results were reported by other authors (23).

Hypertension is present in 50-80% of patients on regular HD (24, 25). The prevalence of hypertension (predialysis blood pressure ≤140/90 mmHg) in our study group was 36.52%. Univariate and multivariate logistic regression analysis showed that high blood pressure, together with other risk factors, contributes significantly to

the development of LVH. Target arterial blood pressure values for patients on regular HD are ≥140/90 mmHg, or ≥160/90 mmHg in elderly patients (24, 25).

Hyperhomocysteinaemia (tHcy >15 μmol/L) is an independent risk factor for atherosclerosis in HD patients (26). Over 80% of HD patients have increased plasma homocysteine. The prevalence of hyperhomocysteinaemia in our study group was 86.09%. A statistically significant positive correlation exists between plasma homocysteine and LVMi in HD patients (26). Univariate logistic regression analysis showed a non statistically significant correlation between homocysteine concentration and LVMi in our study group (R=0.0001, p=0.1600).

Inflammation (CRP >5.0 mg/L) is present in 30-50% of patients on regular HD (27). In our study group, microinflammation was present in 34.78% of patients. Some authors have reported a statistically significant positive correlation between CRP concentration and LVMi (28), but our results have not confirmed a statistically significant positive correlation between CRP levels and the echocardiographic parameters of LVH (R=0.0001, p=0.6559).

Hyperlipidaemia is an independent risk factor for atherosclerosis in HD patients (2). Hypertriglyceridaemia and low HDL-cholesterol are present in 30 - 50% of HD patients (2). In our study group, HDL-cholesterol <1.0 mmol/L was present in 20% of patients. Multivariate logistic regression analysis showed that decreased HDL-cholesterol together with other risk factors contributes to LVH development.

Secondary hyperparathyroidism (SHPTH) is often present in HD patients (19). The prevalence of SHPTH (iPTH >500 pg/mL) in our study group was 20%. No statistically significant correlation was found between serum iPTH and LVMi in our study group (R=0.0001, p=0.8884).

Left ventricular hypertrophy is an exceedingly frequent complication and represents the strongest predictor of adverse cardiovascular events in HD patients (3, 4). An increase of LVMi ≥1.0 g/m2/month is associated with an increased risk of cardiovascular complications (30). In patients with normal LV volume (iEDV ≤90 mL/m2) and systolic function (LVFS >25%, LVEF >50%), LVMi >120 g/m2 and LVMi/iEDV >2.2 g/mL are independently associated with late mortality (mortality rate >2 years following the start of regular HD treatment). Patients on chronic HD with LV volume >120 mL/m2 and LVMi/EDVi <1.8 also have a high cardiovascular mortality risk (31-33). Patients in our study group with LVMi >120 g/m2 and iEDV >90 mL/m2 had a significantly higher all-cause mortality rate then patients with LVMi ≤120 g/m2 and iEDV ≤90 mL/m2. Furthermore, patients with LVMi >120 g/m2 and iEDV ≤90 mL/m2 had a significantly higher cardiovascular mortality rate then patients with LVMi ≤120 g/m2 and iEDV ≤90 mL/m2. Other authors reported similar findings, suggesting that LV remodelling significantly influences outcome in HD patients (32, 33). Patients with LVH (LVMi >125 g/m2) have a significantly higher five-year mortality rate then patients with LVMi <125 g/m2 (34). Left ventricular hypertrophy is an independent predictor of cardiovascular mortality in patients on regular HD (34).

Echocardiographic assessment of LV remodelling enables identification of patients with increased risk for cardiovascular complications. Determining the most sensitive parameters for identifying patients at risk for cardiovascular complications enables timely and adequate treatment, thus providing a higher survival rate and better quality of life for HD patients (35-43).

ABBREVIATIONS:

HD - hemodialysis
LVH - left ventricular hypertrophy
LVMi - left ventricular mass index
LVEDVi - left ventricular end-diastolic volume index
QAV - arteriovenous fistula blood flow
cTnT - cardiac troponin T
cTnI - cardiac troponin I
PCD - percutaneous implantation of cardioverter defibrillator
MAP - mean arterial blood pressure
iPTH - intact parathyroid hormone
Ca x PO4 - calcium-phosphorus product
IVSd - interventricular septal wall thickness in diastole
LVPWd - left ventricular posterior wall thickness in diastole
LVEDD - left ventricular end-diastolic diameter
LVESV - left ventricular end-systolic volume
LVEDV - left ventricular end-diastolic volume
LVFS - left ventricular fractional shortening
LVEF - left ventricular ejection fraction
ESRD - end-stage renal disease
SHPTH - secondary hyperparathyroidism
BMI - body mass index
Kt/Vsp - dialysis adequacy index
BP - blood pressure
MAP - mean arterial blood pressure
IDWG - interdialysis weight gain
CRP - C-reactive protein
GN - glomerulonephritis
PN - pyelonephritis

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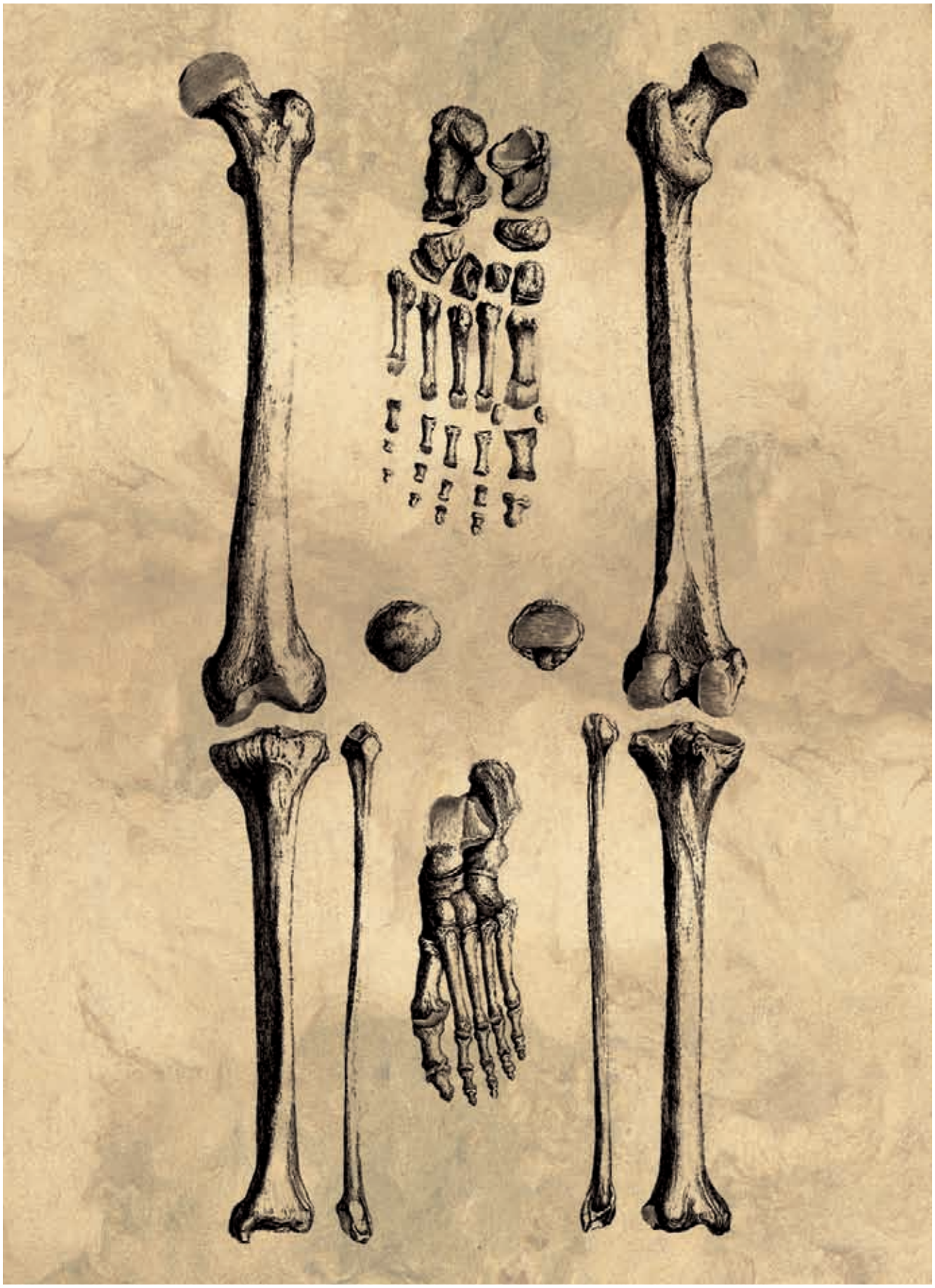

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CHROMOGRANIN A TISSUE EXPRESSION AS A PROGNOSTIC FACTOR IN ADVANCED NON SMALL CELL LUNG CANCER

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TKIVNA EKSPRESIJA HROMOGRANINA A KAO PROGNOSTIČKI FAKTOR KOD ODMAKLOG NESITNOĆELIJSKOG KARCINOMA PLUĆA

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ABSTRACT

To determine the frequency of chromogranin A (CgA) and influence on survival of treated patients with advanced non small cell lung cancer (NSCLC). This study included 236 patients with histological diagnosis of advanced NSCLC (III and IV disease stage). Combined chemotherapy and radiotherapy protocol was used in III stage of disease (without pleural effusion) where as chemotherapy was used in III stage (with pleural effusion) as well as in IV stage of disease. Immunohistochemical analysis of CgA tissue expression was determined in tissue assays using antibodies to CgA. The overall survival of patients was assessed in one year and two years follow – up period. Of 236 eligible patients, 36 (15,25 %) had CgA expression. Squamous cell lung carcinomas had the least frequency of CgA tissue expression (8,7%). The 1-year and 2-year survival rates were 64% and 27% in group of patients with CgA expression compared to 32% and 6% in group without CgA expression (log-rank test:$p<0.001$). The median survival time in group of patients with and without positive CgA expression was 15.7 vs 12.3 months, respectively. One year survival rate was higher in NSCLC patients with more than 50% of CgA positive cancer cells (log-rank test: $p<0.001$).

Key words: non small cell lung cancer, neuroendocrine expression, chromogranin A, frequency, survival

SAŽETAK:

Ispitivana je učestalost hromogranina A (CgA) i njegov uticaj na preživljavanje kod lečenih bolesnika sa odmaklim nesitnoćelijskim karcinomom pluća. U studiju je uključeno 236 bolesnika sa histološkom dijagnozom NSCLC (III i IV stadijum bolesti). Kombinovana hemio i radioterapija bila je uključena u III stadijumu bolesti (bez pleuralnog izliva), a samo hemioterapija u III (sa pleuralnim izlivom) i IV stadijumu bolesti. Za imunohistohemijsku analizu tkivne ekspresije hromogranina A korišćena su mišja, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodi{njem periodu. Od ukupno 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije CgA (8,7%) imao je skvamocelularni ksarcinom pluća. Jednogodišnje i dvogodišnje preživljavanje bilo je 64% i 27% u grupi pacijenata sa ekspresijom CgA u poređenju sa 32% i 5 % u grupi bez ekspresije CgA ((log-rank test:$p<0.001$). Srednje vreme preživljavanja u grupi pacijenata sa i bez ekspresije CgA bilo je 15,7 odnosno 12,3 meseca. Jednogodišnje preživljavanje bilo je veće u grupi pacijenata sa više od 50% pozitivnih CgA tumorskih ćelija (log-rank test: $p<0.001$).

Ključne reči: nesitnoćelijski karcinom pluća, neuroendokrina ekspresija, hromogranin A, učestalost, preživljavanje

1. INTRODUCTION

Lung cancer is the leading cause of cancer death in the world. Non-small cell lung carcinoma (NSCLC) accounts for about 80% of all lung cancers. A high level of chemotherapy and radiotherapy resistence is described in non small cell lung cancer but 5-year overall survival rate was only 14% (1). Differing survival outcomes among patients within a stage suggests the existence of other tumor factors affecting prognosis (2). In the past two decades there have been substantial changes in concepts regarding the nature of lung tumors showing neuroendocrine (NE) differentiation (3). Immunohistochemistry (IHC) is the most practical method of assessing protein expression changes in histopathology. IHC not only provides a semiquantitative assessment of protein abundance but also defines the cellular localisation of expression. These considerations have led to the extensive use of IHC in studies on prognostic markers for tumors (2).

IHC studies indicated that NE features are expressed by 10-30% of ordinary NSCLC (4, 5, 6) especially adenocarcinomas, large cell carcinomas and squamous cell carcinomas, all traditionally considered of non-

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NE nature.These tumors are referred to collectively as NSCLC with NE differentiation (NSCLC-NE). They are characterized by panendocrine expression, neuroamins, neuropeptids and have ultrastructural pattern of specific secretory granules confirmed using immunohistochemical method or by electronic microscopy (7). Clinical and therapeutic significance NSCLC-NE has not been firmly established (3).

Recent studies in which neuroendocrine expression was described as prognostic factor, have provided inconsistent and sometimes conflicting results. Some series have shown NSCLC-NE to be associated with longer survival (5,8), whereas others have not (9,10). Carnaghi et al. confirmed these controversial data, analizing 13 large clinical trials. In two of them, authors described shorter survival, in eight studies expression did not correlate with survival, but in rest three there were significantly longer survivals (11).

Chromogranins are the major proteins in peptide containing dense core (neurosecretory) granules, and antibodies against these are the most specific markers of NE differentiation (6). Chromogranin A (CgA) is a high molecular weight acidic glycoprotein originally isolated from adrenal medulla. It is released along with neuroendocrine peptides through exocytosis from dense-core neurosecretory granules and its detection is directly correlated with the presence of these neurosecretory granules (12). It has been found that a broad spectrum of immunohistochemical markers can highlight neuroendocrine (NE) differentiation in lung tumors, although CgA remain the most strikingly consistent general marker due to its close correlation with the ultrastructural evidence of neurosecretory granules and small clear vesicles, respectively.

The goal of the current study was to determine frequency and influence of CgA expression on survival of treated patients with advanced NSCLC.

2. PATIENTS AND METHODS

2.1. Patients

The study included 236 patients with histological stage III-IV NSCLC, diagnosed and treated at Military Medical Academy, Belgrade and Clinical Center Kragujevac between January 2001 and December 2006. The disease was classified according to the revised International System for Staging Lung Cancer (13). Staging was performed prior to the most recent update of the therapy protocol. Therapy was determined according to disease stage. Patients with IIIA and IIIB stage of disease (without pleural effusion) were treated with combined chemotherapy and radiotherapy. Combined cysplatin-carboplatin chemotherapeutical protocol (not more than six cycles) was conducted until the disease progression (increase more than 20% in measurable tumor). When the progression was noted, the treatment was continued with radiotherapy only (Split course, TD 55-60Gy). Patients with IIIb (with pleural effusion) as well as with IV stage of disease were treated only with chemotherapy. Survival of treated patients was assessed at 1-year and 2-year follow up period.

2.2. Histology and immunohistochemistry

Formalin-fixed and wax-embedded tumor tissues was cut into 4 μm-thick sections and mounted on slides (Super Frost® Plus, Braunschweig, Germany). Regular hematoxylin and eosin (H&E) staining was used for classification according to the WHO classification system for lung carcinoma (14).

Sections for IHC were dewaxed with xylene, rinsed in graded alcohol, rehydrated in water, and immersed in 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Antigen retrieval was achieved by heating the sections in a microwave (Panasonic NN-252W) for 20 min in 0,5M citrate buffer (pH 6.0). The sections were incubated with the anti-human CgA (1:100, M0869, DakoCytomation, Glostrup, Denmark) for 20 h at 4 } C. Between each step the sections were washed in TBS with 0.05% Tween 20, and the immunoreactivity was visualized using Envision® (K5007, DakoCytomation, Glostrup, Denmark) . A pancreatic tissue was used as positive control.

Assessments of staining intensity (0 = none, 1+ = weak, 2+ = moderate, 3+ = strong) and percentage of tumor cells positive (0 = none, 1+ = <10%, 2+ = 10-50%, 3+ = >50%,) were made (15). For CgA antibody the score for intensity was multiplied by that for distribution to give an intensity-distribution (ID) score. An ID score of >2 was used as the criterion for evidence of CgA tissue expression.

2.3.Statistics

Patients' overall survival time was defined as the interval from date of diagnosis to death or to last contact for living patients. Overall survival was graphically presented using Kaplan–Meier method. The log-rank test was used to analyse patients' survival data between groups. Median overall survival time and the 1-year survival rate were obtained from the Kaplan–Meier curves. The Chi-square ($\chi 2$) test was used to compare differences in patients' characteristics between groups. The level of statistical significance was defined as $p<0.05$.

3.RESULTS

3.1. Patient's characteristics

A total of 236 patients with advanced non small cell lung cancer were examined. The patient's characteristics were shown in table 1. The median age of the patients was 62.35±11.57 years (SD), range 37-74. The majority of them (42.41%) belonged to 60-69 years cohort group. One hundred and seventy four (73,72%) of the patients

were male and 62 (26.28%) were female. At diagnosis, fourty eight patients were pathologically staged as IIIA disease (20,36%), 78 (33,05%) as IIIB disease without pleural effusion, 33 (18.22%) with pleural effusion and 67 (28,37%) had metastases (stage IV disease).

Table 1. Patient's characteristics

Patients' characteristics	No. of patients
Total	236
Median age (years)	62
Range of years	37-74
Gender	
Male	174 (73,72%)
Female	62 (26,28%)
ECOG PS	
0	81 (34,32%)
1	77 (32,63%)
2	78 (33,05%)
Stage	
IIIA	48 (20,36%)
IIIB without pleural effusion	78 (33,05%)
III B with pleural effusion	43 (18,22%)
IV	67 (28,37%)
Histology	
Squamous cell carcinoma	115(48,72%)
Adenocarcinoma	77(32,63%)
Adenosquamous carcinoma	29 (12,29%)
Large cell carcinoma	15 (6,36%)

Histologically, one hundred and fifteen (48,72%) were classified as squamous cell carcinoma, 77 (32.63%) were adenocarcinomas, 15 (6.36%) were large cell carcinomas and 29 (12,29%) were adenosquamous carcinomas.

3.2. Chromogranin A characteristics

Of 236 eligible patients, 36 (15,25 %) had CgA expression. Distribution of CgA expression in relation to NSCLC histological types is demonstrated in table 2.

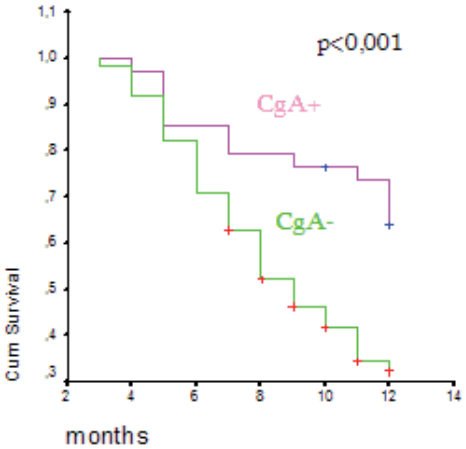
Table 2. CgA expression distributio in relation to NSCLC histological type

CgA neuroendocrine expression		
NSCLC histological types	**positive** n (%)	**negative** n (%)
Squamous cell carcinoma	10 (8,70%)	105 (91,3%)
Adenocarcinoma	16 (20,78%)	61 (79,22%)
Large cell carcinoma	5 (33,3%)	10 (66,7%)
Adenosquamous carcinoma	5 (17,24%)	24 (82,76 %)

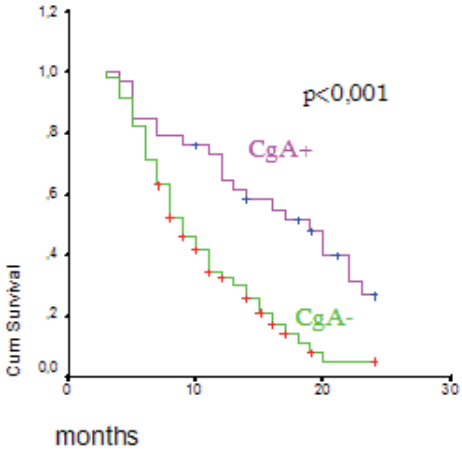
Of 115 patients with squamous cell carcinomas only 10 (8,7%) had CgA expression. The frequencies of CgA expression in group of patients with adenocarcinomas, large call carcinomas and adenosquamous carcinomas were 20,78%, 33,3% and 17,24%, respectively. There was a positive correlation between CgA expression and NSCLC histological types ($p<0.001$).

3.3. Survival rate analysis

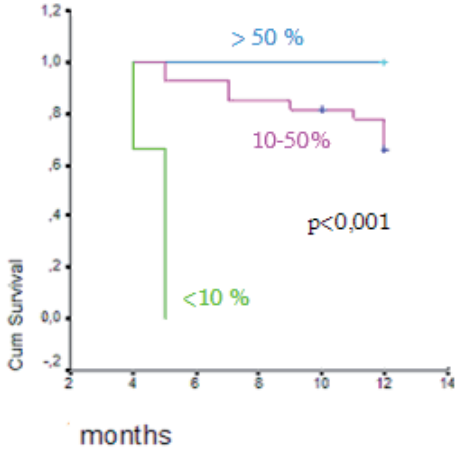
The 1-year survival rate was 64% in group of patients with CgA tissue expression compared to 32% in group without CgA tissue expression (Graph 1). The 2-year survival rate was 27% in group of patients with CgA tissue expression compared to 6% in group without CgA tissue expression (Graph 2). There was a positive correlation between survival rate of treated patient and CgA tissue expression in the 1-year (log-rank test: $p< 0.001$) and 2-year follow-up period (log-rank test:$p< 0.001$). Patients with a CgA expression had a median overall survival time longer than those without this neuroendocrine marker expression, 15.7 versus 12.3 months, respectively.



Graph 1. The 1-year survival rate curve of NSCLC patients with CgA positive and negative tissue expression



Graph 2. The 2-year survival rate curve of NSCLC patients with CgA positive and negative tissue expression



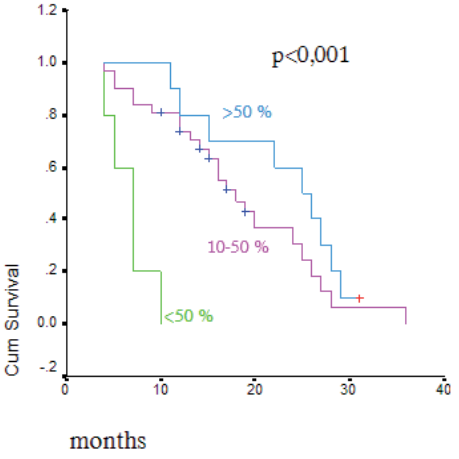
Graph 3. The 1-year survival rate curve of NSCLC patients with neuroendocrine expression in relation to percentage of CgA positive cancer cells

Multivariate binary logistic regression was used to assess simultaneous influence of all parameters to median survival time. (table 3). Parameters which are proved to have influence on median survival time in patients with NSCLC of stage III and IV are stage of disease (p<0.001) and percentage of positive tumor cells (p=0.000).

Table 3. Influence of parameters on survival time in patients with NSCLC

	Parameter	*P*
First step	Sex	0,238
	Age	0,436
	Hystological type of tumor	0,137
	Diferentiation level	0,087
	Loss of body weight	0,741
	Expression of Chr A	0,381
	Chemotherapeutic protocol	0,807
	Stage of disease	**0,017**
	Karnoffsky index	0,412
	LDH	0,239
	Therapeutic regimen (HT ± RT)	0,154
	Percentage of positive tumor cells	**0,034**
	Constant	0,201
Last step	**Stage of disease**	**0,002**
	LDH	0,065
	Percentage of positive tumor cells	**0,000**
	Constant	0,033

The 1-year survival rate of patients with more than 50% of CgA positive cancers cells was 100%. Survival time between patients with less than 10% of CgA positive cancer cells was no longer than 5 months (Graph 3). There was significant difference in the 1-year (Graph 3) and 2- year (Graph 4) survival time and percentage of CgA positive cancer cells (log-rank test:p<0.001).



Graph 4. The 2-year survival rate curve of NSCLC patients with neuroendocrine expression in relation to percentage of CgA positive cancer cells

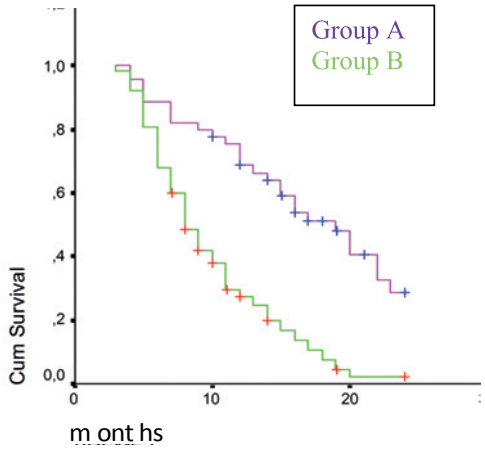
Analysis of one-year and two-year median survival time in patients group A (III A + III B stage, without pleural effusion) and group B (III B with pleural effusion and IV stage) showed statistically significant difference in survival (p = 0.000). One-year (62%) and two-year (30%) median survival time was longer in patients group A compared to group B, where 38% of patients lived one year and more, but only 5% of treated patients lived for 24 months (Graph 5).

4. DISCUSSION

The aim of the present investigation was to analyse frequency of chromogranin A tissue expression and impact of this strikingly consistent neuroendocrine marker on survival of treated patients with advanced non small cell lung cancer.

For this purpose, expression of CgA, which is strongly associated with neuroendocrine differentiation in NSCLC

(6,16) was assessed in the primary non small cell lung tumors.



Graph 5. Two-year survival in relation to stage of disease

One of the problems is the lack of a definition or "gold standard" of NE differentiation.

Differences in tissue processing, tehniques or markers used for highlighting NE differentiation, definitions of positive results and study population selection may account for these contradictory results reported in the clinical meaning of NE differentiation in patients with NSCLC (8,10,17).

Investigating the NSCLC with neuroendocrine differentiation through the expression of neuroendocrine markers, Sorhaug et al. (18) suggested that the frequency of CgA tissue expression was fluctuated from 5% when conventional immunohistochemical method (IHC) was applied to 25% in tyramide signal amplification (TSA) method. For diagnostic purposes, IHC method for detection of specific NE cell components was most often used.

In our study, CgA tissue expression assessed by standard immunohistochemical method was observed in 15,25% of cases. Results from recent series in which the same method was used, suggested that CgA tissue expression was not only depended upon the tehniques used in. In these series, the frequency of CgA tissue expression ranged from 0.0% to 34.4% (5, 9,10,16,19, 20,21).

We found a highly significant positive correlation between histological types of non small cell lung cancer and CgA tissue expression ($p<0.01$). Squamous cell lung cancers showed the least percentage of CgA tissue expression (8,7%), whilst other types of carcinomas had higher frequency of CgA expression. Results from our study were correlated with the results of clinical trials adjusted by other investigators. Pelosi et al. (22) suggested that squamous cell lung cancers showed very low CgA expression frequency (11.5%). According to histology, immunoreactivity for CgA was seen in two of the squamous cell carcinomas (2/9), two of the adenocarcinomas (2/9), and one of the large cell carcinomas (1/2) (18). A considerable overlap occurred in all histological groups, a finding which contrasts with those of other workers who found that NE markers were more commonly expressed in adenocarcinomas and only rarely in squamous cell carcinomas (23).

In the present study, we investigate the impact of CgA expression on prognosis, indicating that NE differentiation is a significant prognostic factor.

In the survival analysis, we found that the presence of CgA tissue expression significantly correlated with survival. The 1-year and 2-year survival rate were significantly higher in group of patients with CgA expression compared to those without expression of this neuroendocrine marker. Median survival time was higher in group of patients with CgA tissue expression compared to those without expression.

Even though some studies have shown a prognostic significance of NE differentiation in subgroups such as adenocarcinomas (17, 22), the present opinion is that the finding of some tumor cells with NE features does not seem to influence prognosis or response to treatment (6, 15, 24).

In a review of the literature, Schleusener et al. (5) reported that NE differentiation in NSCLC has been shown in different studies to be associated with either: improved survival (mostly in chemotherapy-treated patients) or decreased survival (mostly in surgically treated patients), or to have no bearing at all on survival.

Using multivariate analysis, Abbona et al. (23) showed that NE differentiation in NSCLC had a negative impact on survival and was predictive of higher disease stage.

Carnaghi et al. reviewed 13 major studies investigating either the prognostic or predictive value of neuroendocrine differentiation in NSCLC. This review showed that there are conflicting results regarding the importance of neuroendocrine differentiation; two studies showed decreased survival, eight showed no correlation with survival, and three showed improved survival (11).

Skov et al. analized percentage of CgA positive cancer cells as a prognostic factor in NSCLC patients. Patients with more than 10% of CgA positive cancer cells have a longer survival time compared to those with less percentage of positive cells (12). In our study, one year survival rate in group of NSCLC patients with more than 50% CgA positive cancer cells was longer compared to group with less than 10% of CgA positive cancer cells.

Further work is needed to assess their usefulness as NE markers and to see whether they might provide additional information on NE differentiation and prognosis.

Obviously, lung cancer remains a frustrating clinical problem, notorious for poor treatment results.

5. Conclusion

Chromogranin A tissue expression was registered in 15,25% of patients with advanced non small cell lung cancer with neuroendocrine differentiation. Large cell lung cancers had the highest frequency of CgA tissue expression compared to other histological cancer types. The 1-year and 2-year survival time were longer in patients with CgA expression. Moreover, longer survival time was often associated with presence of more than 50% of chromogranin A positive cancer cells. Therefore, CgA tissue expression was associated with improved patient's survival.

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NITRIC OXIDE AND IFN-γ PLASMA LEVELS IN PATIENTS WITH ATOPIC DERMATITIS

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KONCENTRACIJE AZOT MONOKSIDA I IFN-γ U PLAZMI PACIJENATA SA ATOPIJSKIM DERMATITISOM

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ABSTRACT

The underlying mechanisms of skin inflammation in atopic dermatitis (AD) are not completely understood but inflammatory cell activation and dysregulated cytokine production appear to play a critical role in pathogenesis of AD. Inducible nitric oxide synthase (iNOS) is expressed by dermal endothelial cells and perivascular inflammatory cells in the atopic skin lesion, suggesting the involement of nitric oxide (NO) in the skin inflammation of AD. Among the proinflamatory cytokines interferon-gamma (IFN-γ) is the most efficient inducer of NO production. The purpose of the study was to examine IFN-γ and NO plasma levels in patients with AD. We have also measured NO production by mononuclear (MN) and polymorphonuclear (PMN) leucocytes in cells culture systems. Seventeen patients with atopic dermatitis and ten healthy volunteers were included in this study. NO plasma levels of patients with AD were significantly increased (p=0.001) as compared to nonatopic controls. No significant difference in NO levels in MN cells cultures of AD patients and nonatopic controls was observed (p=0.083). NO levels in PMN cells cultures of AD patients were significantly higher (p=0.011). IFN-γ plasma concentration in AD patients was significantly increased as compared to nonatopic controls (p=0.005). Our results suggest that PMN leucocytes in AD patients could be source of increased NO plasma levels in patients with AD. As our patients have lasting eczematous skin lesions, our results also lend support to the two-phase-model for the pathogenesis of AD were in a second phase expresion of Th-1 cytokines, such as IFN-γ, predominates.

Key words: atopic dermatitis, nitric oxide, interferon-gamma.

SAŽETAK

Mehanizmi koji dovode do inflamacije kod atopijskog dermatitisa (AD) nisu u potpunosti razjašnjeni ali se smatra da aktivacija inflamatornih ćelija i poremećena produkcija citokina igraju ključnu ulogu u patogenezi ovog oboljenja. Inducibilna azot monoksid sintetaza (iNOS) je eksprimirana u dermalnim endotelnim ćelijama i perivaskularnim zapaljenskim ćelijama u okviru kožnih lezija, sugerišući ulogu azot monoksida (NO) u inflamaciji kod AD. Interferon gama (IFN-γ) je najefikasniji proinflamatorni citokin u indukovanju produkcije NO. Cilj ove studije je bio da ispita koncentracije IFN-γ i NO u plazmi pacijenata koji boluju od AD. Takođe je određivana i produkcija NO u ćelijskoj kulturi mononuklearnih (MN) i polimorfonuklearnih (PMN) leukocita. Sedamnaest pacijenata i deset zdravih volontera su bili uključeni u studiju. Koncentracija NO u plazmi pacijenata je bila značajno povišena (p=0.001) u poređenju sa istim parametrom kod kontrolne grupe. Nije bilo statistički značajne razlike u koncentraciji NO u supernatantu kultura MN leukocita kod pacijenata u odnosu na kontrolnu grupu (p=0.083). Produkcija NO u kulturama PMN leukocita pacijenata je bila statistički značajno povećana (p=0.011) u poređenju sa kontrolnom grupom. Koncentracija IFN-γ u plazmi pacijenata bila je značajno viša u odnosu na zdrave kontrole (p=0.005). Naši rezultati govore u prilog tome da PMN leukociti pacijenata sa atopijskim dermatitisom mogu biti izvor povišenih vrednosti NO u plazmi. Obzirom da su pacijenti uključeni u ovu studiju imali dugotrajne ekcematozne promene na koži naši rezultati govore u prilog dvofaznog modela patogeneze AD po kome u sekundarnoj fazi toka bolesti predominira ekspresija Th-1 citokina, pre svega IFN-γ.

Ključne reči: atopijski dermatitis, azot monoksid, interferon gama.

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease of unknown aetiology, characterized by typically distributed eczematous skin lesions with lichenification, pruritic excoriations, dry skin and a susceptibility to skin infections (1). A complex interrelationship of genetic, environmental, skin barrier, pharmacological, psychological and immunological factors plays an important part in the pathogenesis of the disease (2). The mechanisms involved in inflammation in AD are not completely clear, but inflammatory cell activation and dysregulated cytokine production appear to play critical roles in the pathogenesis of AD (1).

Controversies still exist regarding the role of the Th2 and Th1 immune system in the pathogenesis of AD (3-8). Skin lesions in AD are characterized by hypertrophy of the dermis and epidermis and infiltration by T

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cells, monocyte-macrophages and eosinophils. Acute skin lesions exhibit increased levels of IL-4 and IL-5 mRNA and protein, suggesting preferential accumulation of Th2 cells (3). Chronic eczematous AD skin lesions contain increased levels of IFN-g mRNA and protein, alone or in combination with IL-4 (9-11), suggesting a switch from an initial Th2 response to a mixed Th1 plus Th2 response. A switch in time from a Th2 to a mixed Th1 plus Th2 response is also observed when patch tests with house dust mite allergen are performed in patients with AD (11). Initially, IL-4 predominates over IFN-g at the site of antigen application, but later the situation is reversed and IFN-g predominates over IL-4 (12). These studies indicate that both Th-cell subsets contribute to the pathogenesis of this disease and suggest that expression of Th1-like and Th2-like cytokines in AD is not mutually exclusive.

The proinflammatory cytokines interferon-gamma (IFN-g), tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are involved in induction of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO). iNOS is expressed by dermal endothelial cells and perivascular inflammatory cells in the atopic skin lesion, suggesting the involvement of nitric oxide in the skin inflammation of AD (13). Among the proinflammatory cytokines, IFN-g is the most efficient inducer of NO production (14).

In our study, we examined IFN-g and NO plasma levels in patients with AD. We also measured NO production by mononuclear (MN) and polymorphonuclear (PMN) leucocytes in a cell culture system.

MATERIALS AND METHODS

Patients and controls

Seventeen patients with AD (8 male and 9 female; aged 5 to 21, mean 12.76±4.74), were included in this study. The diagnosis was based on the criteria of Hanifin & Rajka (19). AD was stable, without recent flare-up; none of the patients was treated with immunosuppressive drugs. AD was graded according to SCORAD (20). The mean SCORAD index was 32.35+14.73. The control group consisted of ten healthy volunteers (6 male and 4 female, aged 6 to 21, mean 13.5±4.45) with negative personal history of atopy.

METHODS

A specimen of peripheral venous blood was collected in the morning. Nitric oxide and IFN-γ levels in the plasma samples were measured. After centrifugation, one part of plasma was used for nitrite extraction and the second part was stored immediately at -20oC until IFN-γ analysis. At the same time, MN and PMN cell cultures were prepared. After 24 h of incubation, culture supernatants were collected and stored at -20oC until use.

NO DETERMINATION

Before testing, the plasma samples were deproteinized by using acid solution. In 1500 μl tubes, 100 μl of 3 M perchloric acid, 400 μl of 20 mM EDTA and 200 μl of plasma were added. Extracts were incubated on ice for 20 minutes, with occasional mixing, and then centrifuged at 1500 rpm for 5 minutes. The supernatants were removed into other tubes and 120 μl 2 M potassium-carbonate was added to neutralize the extracts. The neutralized extracts were stored at -20oC until testing. Immediately before use, extracts were defrosted and centrifuged in order to reduce the presence of potassium-perchlorate particles.

Nitrite (NO2-) is a stable product of NO metabolism that reacts with Griess reagent to create a pink colour. Plasma nitrite levels were measured by spectrophotometric assay as described by Miranda et al. (21). We also used this assay to measure nitrite levels in MN and PMN cell culture supernatants. Griess reagent was prepared just before the experiment by mixing equal amounts of stocks: 2% (w/v) sulfanilamide dissolved in 5% HCl and 0.1% (w/v) aqueous solution of N-1-naphthyl-ethylenediamine-dihydrochloride (N-NEDA). Nitrite solutions in H20 (10 mM) were prepared fresh daily. The experiment was performed at room temperature. The nitrite standard solution was serially diluted (100-1.6 μl) in a 96-well, flat-bottomed, polystyrene microtiter plate in final volume of 100 μl. After loading the plate with plasma samples (100 μl), Griess reagent was added to each well. Distilled water and Griess reagent were used as the standard blank. The absorbance was measured at 540 nm (Multiplate reader 230S, Organon) following 30 minutes of incubation. Nitrite concentration was determined by using Xia software for data analysis, based on the standard curve that was obtained by linear regression absorbance values for each standard (reduced for blank values). Results were expressed as nanomoles per millilitre (nmol/ml).

MN AND PMN CELL CULTURE PREPARATION

MN and PMN leucocytes were obtained from venous peripheral blood according to a widely accepted method by Boym (22). We prepared MN (1x106/ml) and PMN (2x106/ml) cell cultures, and incubated them for 24 h in RPMI 1640 medium with 200IJ penicillin and 200 mg/ml streptomycin, at 370C in an atmosphere of 5% CO2. After incubation was finished, supernatants were collected and stored at -20oC until use. Just before use, supernatants were defrosted and centrifuged in order to remove any residual cells.

NO DETERMINATION IN CELL CULTURES

NO production in MN (1x106/ml) and PMN (2x106/ml) cell cultures was measured indirectly by measuring NO concentration in culture supernatant. NO levels were measured by quantifying nitrite concentrations as described previously, based on a standard curve (RPMI 1640 medium was used as standard blank instead of distilled water).

IFN-γ DETERMINATION

We measured the levels of IFN-γ in the plasma of patients with AD and in controls, using a commercial enzyme-linked immunosorbent assay (HUMAN IFN-γ Elisa kit II BD Biosciences, Pharmingen, San Diego, CA, USA), according to the manufacturer's instructions. Results were expressed as pg/ml.

STATISTICAL ANALYSIS

All values are expressed as mean±standard deviation (X±SD) and median. Commercial SPSS (Statistical Package for the Social Sciences) version 11.0 was used for statistical analysis. Normal distribution of data was tested by using the Kolmogorov-Smirnov test. Statistical evaluation was performed with the nonparametric Mann-Whitney U-test and Kruskal~-Wallis test for unpaired data and Student´s t-test for paired data. A P value of<0.05 was considered to be significant and highly significant when <0.01.

RESULTS

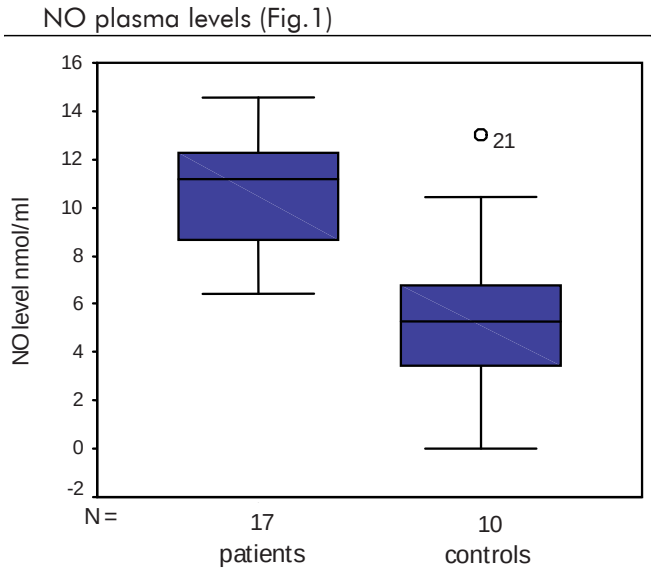

Figure 1. NO plasma levels

NO plasma levels of patients with AD were significantly increased as compared to nonatopic controls (t-test; p=0.001). Mean concentration of NO in plasma of AD patients was 10.46±2.38, while the same parameter in plasma of healthy controls was 5.87±3.7.

NO levels in MN (Fig.2) and PMN (Fig.3) culture supernatant

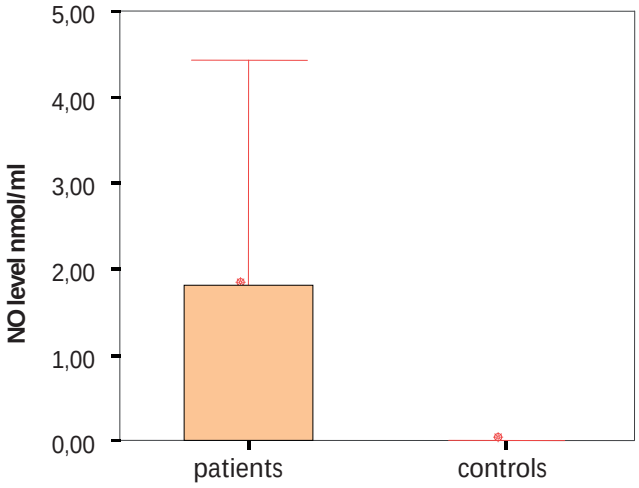

Figure 2. NO levels in MN culture supernatant

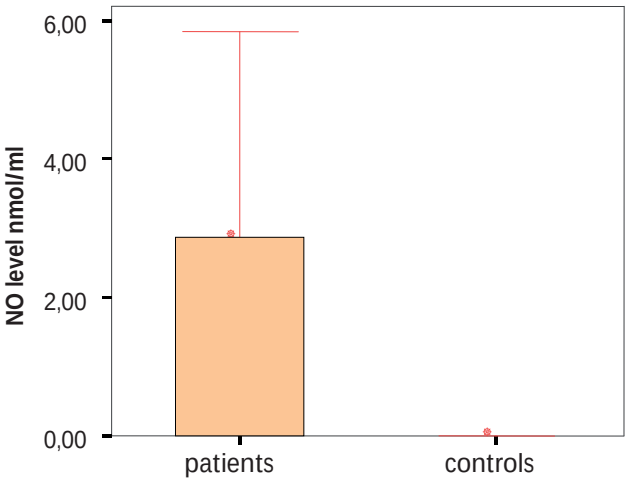

Figure 3. NO levels in PMN culture supernatant

No significant difference in NO levels was observed between MN cultures of AD patients and nonatopic controls (Mann Whitney U test; p=0.083). The mean concentration of NO in MN cultures of AD patients was 1.82±2.61, median=0 (more than 50% of all measured values were 0). The same parameter in MN cultures of healthy controls was too low to be measured.

The NO levels in PMN cultures of AD patients were significantly higher as compared to healthy controls (Mann Whitney U test; p=0.011) The mean concentration was 2.88±2.97 and median 2.49, while the same

parameters in PMN cultures of healthy controls were too low to be measured.

IFN-γ plasma levels (Fig.4)

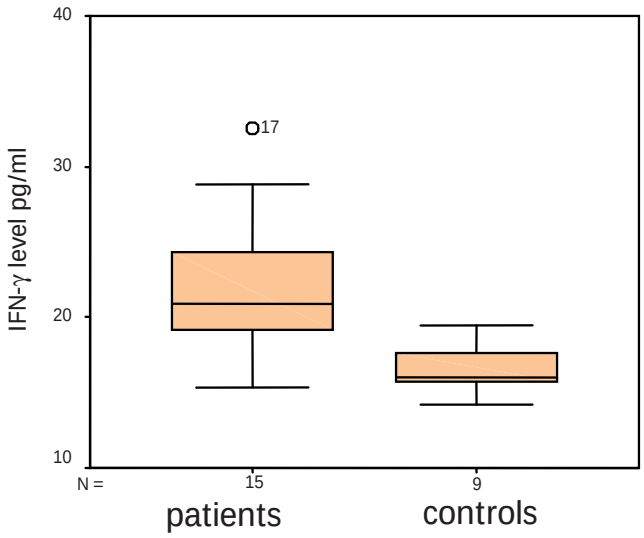

Figure 4. IFN-γ plasma levels

There was a significant difference between IFN-γ plasma concentration in AD patients and in healthy controls (t-test: p=0.005). IFN-γ plasma concentrations in AD patients (21.77±4.85) were significantly increased as compared to nonatopic controls (16.56±1.61).

DISCUSSION

Although the mechanisms involved in inflammation in AD are not completely clear, inflammatory T-cell activation and dysregulated cytokine production appear to play a critical role in pathogenesis of AD (1). As T-cells are potent producers of a large variety of cytokines, several studies have been performed to investigate the cytokine pattern in AD (4-11). These studies indicate that both Th2- and Th1-type cytokines contribute to the pathogenesis of skin inflammation. Grewe et al. (3,10) demonstrated in situ expression of Th1-like (IFN-γ) and Th2-like (IL-4) cytokines in lesional AD skin, indicating that eczematous skin lesions are not the result of exclusive expression of either Th1-like or Th2-like cytokines. They proposed a two-phase-model for the pathogenesis of AD. Development of AD skin lesions results from sequential activation of Th cells: in an early phase, Th2-like cytokines are crucial for initiation of atopic eczema, and in a second phase, expression of Th1-like cytokines (such as IFN-γ) predominates. The predominance of IFN-γ-producing T-cells is responsible for the chronicity of AD lesions and determines the severity of disease.

Few published studies examine levels of IFN-γ in serum. Aleksza et al. (15) measured the levels of circulating cytokines (IFN-γ, IL-4, IL-10 and IL-13) in serum of AD patients and healthy controls. The levels of all cytokines were elevated in patients with AD, but significant differences was found only for IL-10 and IL-13. Niwa (16) assessed cytokine levels in both plasma and serum from the patients with AD and healthy volunteers and found that IL-2, IL-5, IL-10 and IFN-γ were significantly elevated in the plasma from AD patients, but not in their serum.

In our study, IFN-γ plasma concentration in AD patients was significantly increased as compared to nonatopic controls. According to the two-phase-model for the pathogenesis of AD, a predominance of IFN-γ-producing T cells is responsible for the chronicity and maintenance of eczematous skin lesions. As our patients have lasting skin lesions, our results also lend support to the two-phase-model for the pathogenesis of AD, in which a second phase involves predominate expression of Th-1 cytokines, such as IFN-γ.

IFN-γ is the most efficient inducer of NO production (14). IFN-γ plasma levels directly determine NO plasma levels, as well as ex vivo NO production by leucocytes. NO has been found to be important in a number of different physiological processes. NO plays an important role in the initiation and progression of atopic diseases such as asthma, hay fever and atopic dermatitis (24,25). Of particular relevance to the skin and atopic dermatitis are the roles of NO in vasodilatation, inflammation, and immunomodulation, as well as oxidative damage to cells and tissues (13).

Taniuchi et al (17) showed increased NO metabolite levels in serum of children, aged 0.4-8 years with AD. These authors also showed a correlation between serum nitrate (NO3-) levels and skin lesion severity. Guzik et al (18) undertook a similar study with adults, aged 18-47 years with AD, but could not confirm the observation of Taniuchi. They postulated that the difference between their observations and findings by Taniuchi et al. could be explained by the fact that the area of affected skin relative to total skin surface and body weight is smaller in adults with AD as compared to children with AD. Tsukahara et al. (23, 24) measured urinary concentrations of nitrite/nitrate in children with exacerbation of AD. They did not find significant differences in those parameters between AD patients and healthy controls. Their results suggest that endogenous NO synthesis in children with exacerbation of AD is similar to that in healthy controls.

Our patients were 5 to 21 years old and blood samples for analysis were taken during relative clinical remission (no significant vasodilation, erythema or oedema). NO plasma levels in our patients were significantly increased as compared to nonatopic controls. Our results suggest that NO plasma concentrations in patients older than 8 years in clinical remission are increased as compared to healthy controls.

No published studies have examined NO levels in MN and PMN cultures of AD patients, preventing com-

parisons of our results with those obtained in similar studies.

NO levels in MN cultures of AD patients included in our study were very low and no significant difference was observed between NO levels in MN cultures of AD patients as compared to nonatopic controls. Those results suggest that mononuclear leucocytes do not represent the cellular source of increased NO plasma levels in patients with AD.

NO levels in PMN cultures of AD patients were low but significantly higher as compared to healthy controls. Our results suggests that PMN in AD patients represent a source of increased NO plasma levels in patients with AD. Other sources such as endothelial cells, keratinocytes, Langerhansŏ cells of the affected area, and their contribution to increased NO plasma levels, should not be overlooked.

At present, there is no treatment directed at the underlying cause of AD. A better understanding of the mechanisms that underlie AD is therefore critical for the design of new and more effective treatments for this common disease. Our results indicate that use of NO pathway modulators might be a potentially useful strategy for the treatment of AD. Also, in further research we plan to examine AD lesional skin for cytokine expression during remission and exacerbation of AD.

ABBREVIATIONS:

AD – Atopic dermatitis,
IFN-γ - Interferon-gamma,
IL – Interleukin,
MN – Mononuclear,
NO – Nitric oxide,
PMN – Polymorphonuclear.

SKRAĆENICE:

AD – Atopijski dermatitis,
IFN-γ - Interferon gama,
IL – Interleukin,
MN – Mononuklearni,
NO – Azot monoksid,
PMN – Polimorfonuklearni.

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THE IMPORTANCE OF EXPANDED SGARBOSSA CRITERIA FOR DIAGNOSIS OF ACUTE CORONARY SYNDROMES IN PATIENTS WITH LEFT BUNDLE BRANCH BLOCK: A CASE SERIES

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ZNAČAJ PROŠIRENIH SGARBOSSA KRITERIJUMA U DIJAGNOZI AKUTNOG KORONARNOG SINDROMA U PACIJENATA SA BLOKOM LEVE GRANE HISS-OVOG SNOPA

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SUMMARY

The occurrence of left bundle branch block (LBBB) significantly deforms the processes of depolarization and repolarization, which decreases the sensitivity of the electrocardiogram (EKG) as a diagnostic method in patients with acute ischemia. The presence of expanded Sgarbossa criteria increases the sensitivity and specificity of the ECG. **Aim:** The objective of this study was to examine the connection of expanded Sgarbossa criteria with acute coronary syndrome (ACS) in patients with LBBB and the intrahospital mortality of these patients with all certain risk factors. **Methodology of the study:** This study included 340 patients hospitalized in the coronary unit of the KC Kragujevac Cardiology Center from 1 January 2008 to 10 January 2008. During this period, 20 patients were diagnosed with left bundle branch block. Patients with LBBB were divided into two sub-groups according to the ranges of cardio-specific enzymes: 14 (70%) had a laboratory confirmed diagnosis of ACS, and 6 (30%) patients did not have a confirmed ACS diagnosis. In this study we observed the presence of the following risk factors: hypertension (HTA), heritage, diabetes mellitus (DM), smoking, hyperlipoproteinemia (HLP), and a previous attack of ischemic heart disease (IHD). Besides the risk factors mentioned above, we observed heart rate, the values of systolic and diastolic blood pressure on admission, the presence of expanded Sgarbossa criteria, the use of medications, and intrahospital mortality. Statistical analysis was performed by Fisher's test or a Student's T-test where appropriate. **Results:** We showed that the expanded Sgarbossa criteria should not be neglected when establishing a diagnosis of acute coronary syndrome. The following criteria were particularly important: positive T wave in leads V_5 or V_6, a sign of Cabrera ascending limb of the S wave in V_3 or V_4, the presence of an S wave in lead V_5 or V_6, the presence of a Q wave in two contiguous precordial leads, and left-axis deviation. We also showed that diabetes mellitus (DM) was the only risk factor linked with ACS with LBBB ($p = 0.018$) because it increased the likelihood of the occurrence of ACS with LBBB (odds ratio [OR] = 42; 95% CI 1.176-1497.973; $p < 0.05$) by 42 times and had a statistically significant influence on the occurrence of ACS with LBBB ($p = 0.04$). We observed that intrahospital mortality was higher if ACS was accompanied by LBBB even though Fisher's test showed no dependency between mortality and patients with ACS and LBBB ($p = 1.00$).

Key words: LBBB, ACS, Sgarbossa criteria, diabetes mellitus, mortality

SAŽETAK

Pojava bloka leve grane (LBBB) Hiss-ovog snopa značajno deformiše proces depolarizacije i repolarizacije miokarda što smanjuje senzitivnost elektrokardiograma (EKG) kao dijagnostičke metode kod pacijenata sa akutnom ishemijom. Prisustvo proširenih Sgarbossa kriterijuma povećava senzitivnost i specifičnost EKG-a.

Cilja rada je da se ispita povezanost proširenih Sgarbossa kriterijuma i akutnog koronarnog sindroma u pacijenata sa LBBB i intrahospitalni mortalitet ovih pacijenata uz prateće faktore rizika.

Metod rada. U studiju je uključeno 340 pacijenta hospitalizovanih u Koronarnoj jedinici Centra za kardiologiju KC Kragujevac u periodu od 01.01.2008 godine do 01.10.2008 godine od kojih je 20 pacijenata sa blokom leve grane Hiss-ovog snopa. Pacijenti sa LBBB su podeljeni u dve grupe zavisno od vrednosti kardiospecifičnih enzima: 14 (70%) je imalo laboratorijski potvrđenu dijagnozu akutnog koronarnog sindroma (ACS) a 6 (30%) pacijenata nije imalo. U našim istraživanjima pratili smo zastupljenost sledećih faktora rizika: hipertenzija (HTA), hereditet, diabetes mellitus (DM), pušenje, hiperlipoproteinemija (HLP) i prethodni atak ishemijske bolesti srca (IBS). Pored navedenih faktora rizika pratili smo srčanu frekvencu, vrednost sistolnog i dijastolnog krvnog pritiska na prijemu, prisustvo proširenih Sgarbossa kriterijuma, upotrebu lekova i intrahospitalni mortalitet. Od statističkih metoda koristili smo: Student-ov T-test, Fisher-ov egzaktni test, senzitivnost i specifičnost.

Rezultati. Pokazali smo da proširene Sgarbossa kriterijume ne treba zanemarivati u postavljanju dijagnoze akutnog koronarnog sindroma, a posebno: pozitivan T talas u odvodima V_5,V_6, Cabrera-in znak-nazubljen S zubac u odvodu V_3 ili V_4, prisustvo S zupca u odvodu V_5 ili V_6, prisustvo Q zupca u dva susedna prekordijalna, levogram. Utvrdili smo da od posmatranih faktora rizika jedino je diabetes mellitus povezan sa ACS u LBBB ($p = 0,018$) jer četrdesest i dva puta povećava šansu za nastanak ACS u LBBB (OR=42; CI 1,176-1,297; 0,973) i statistički značajno utiče na pojavu ACS u LBBB ($p = 0,04$). Prmetili smo da je intrahospitalni mortalitet veći ako je ACS udružen sa LBBB, međutim Fisher-ov test je pokazao da ne postoji zavisnost između inrahospitalnog mortaliteta i pojave ACS kod pacijenata sa LBBB ($p = 1,00$).

Ključne reči: LBBB, ACS, Sgarbossa kriterijumi, diabetes mellitus, mortalitet

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INTRODUCTION

Complete left bundle branch block (LBBB) has been defined as a duration of QRS complex longer than 120 msec, Q or rS in V_1, an absence of Q in D_1, aVL, V_5, V_6, peak of R wave on 60 msec in D_1, aVL, V_5, V_6, negative T in D_1, aVL, V_5, V_6, positive T in V_1, V_2. The presence of LBBB significantly deforms the processes of myocardial depolarization and repolarization, which decreases the sensitivity of the electrocardiogram (ECG) as a diagnostic method in patients with acute ischemia. In LBBB, normal septal Q waves disappear, which are present in left precordial leads (V_5, V_6) as well as in the leads D_1 and aVL, where during the regular implementation they have been detected due to initial depolarization of septum from left to right. Instead, septum has been depolarized by impulses that come from the right bundle branch block (RBBB) and therefore its depolarization goes from right to left, providing immediately R wave in the left leads. The presence of a Q wave in leads D_1, aVL, V_5, V_6 in the LBBB, regardless of how small they are, is considered to be pathological and signifies myocardial injury (1). A diagnosis of acute coronary syndrome (ACS) in the presence of LBBB can be obtained using the expanded Sgarbossa criteria (Table 1) (2).

Table 1. Expanded Sgarbossa criteria

1. Concordant with QRS complex ST segment elevation>1mm
2. Disconcordant with QRS complex ST segment elevation>5mm
3. Depression of ST segment in the leads V_2, V_3 >1mm
4. Presence of Q waves in contiguous precordial leads or in two limb leads
5. R wave regression from leads V_1 to V_4
6. QS patterns from leads V_1 to V_4 and rS in V_1
7. Positive T wave in leads V_5, V_6
8. Sign of Cabrera ascending limb of the S wave in V_3 or V_4
9. Sign of Chapman (ascending limb of R waves in lead I, aVL, V_5 or V_6)
10. Presence of S wave in lead V_5 or V_6
11. Left-axis deviation

AIM

In this study, we evaluated the presence of risk factors within patients, heart rate, values of systolic and diastolic blood pressure on admission, the presence of expanded Sgarbossa criteria, the administration of medications, and intrahospital mortality.

The study was designed to examine the link between expanded Sgarbossa criteria and patients with acute coronary syndrome in the presence of LBBB and intrahospital mortality with the following risk factors: hypertension, heritage, diabetes mellitus, smoking, hyperlipoproteinemia, and a previous attack of ischemic heart disease.

METHODS

Three hundred forty patients entered this study. All were hospitalized at the Coronary unit of the Cardiology Center, Clinical Center "Kragujevac". The mean follow-up was nine months (starting from 1 Jan 2008 to 10 Jan 2008). A total of 20 patients had LBBB. LBBB of the His bundle was diagnosed based on standard electrocardiographic criteria. A diagnosis of ACS was established by the presence of typical precordial pain for a duration longer than 20 minutes and the presence of electrocardiographic expanded Sgarbossa criteria and was confirmed by a finding of high cardio-specific markers (creatine kinase double reference ranges, troponin >0,1 μg/l). LBBB was assumed to be de novo in the absence of anamnestic data or previously documented LBBB. Patients with LBBB were divided into two subgroups according to ranges of cardio-specific enzymes: 14 (70%) had a laboratory confirmed diagnosis of ACS (ranges of cardio-specific markers above the reference ranges), and 6 (30%) patients did not have diagnosed ACS (cardio-specific markers in the reference range).

In this study we observed the presence of the following risk factors: hypertension (HTA), heritage, diabetes mellitus (DM), smoking, hyperlipoproteinemia (HLP), and a previous attack of ischemic heart disease (IHD). Besides the risk factors mentioned above, we observed heart rate, the values of systolic and diastolic blood pressure on admission, the presence of expanded Sgarbossa criteria, the use of medications, and intrahospital mortality. Distribution data were performed using the Shapiro-Wilk test. Statistical analysis was performed by Fisher's exact test or Student's T-test where appropriate.

RESULTS

The average age of patients with LBBB was 60 years, and the sex of observed patients with LBBB was as follows: 14 of the (70%) male sex (11 patients with ACS and 3 without ACS) and 6 of the (30%) female sex (3 patients with ACS and 3 without ACS).

The rate of the following risk factors are shown in figures 1 and 2: hypertension (HTA), heritage, diabetes mellitus (DM), smoking, hyperlipoproteinemia (HLP), and a previous attack of ischemic heart disease (IHD) related to the sex of patients with or without ACS and LBBB.

p values were 1.00 (HTA), 0.336 (Heritage), 0.018 (DM), 1.00 (Smoking), 0.325 (HLP), and 1.00 (Previous attack of IHD) (Fisher Exact´s Test).

The systolic blood pressure of all patients with LBBB was 128.28 ± 30.96 (ACS) and 148 ± 29.2 (without ACS) (p = 0.202; Student's T-test). The diastolic blood pressure of all patients with LBBB was 79.5±14.88 (ACS) and 79.6±11.94 (without ACS) (p = 0,981; Student's T-test). The results are summarized in figure 4.

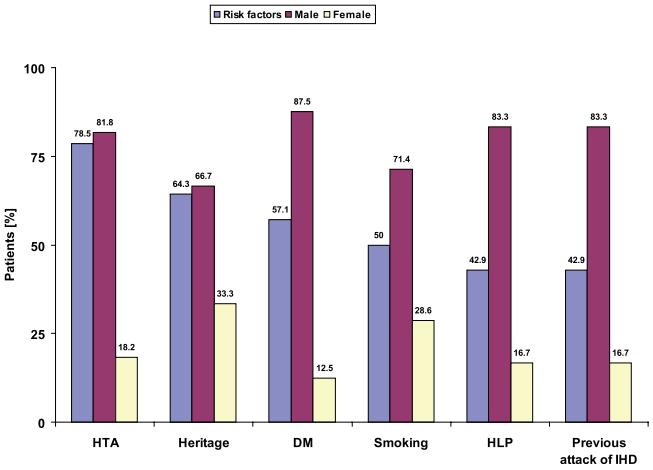

Figure 1. Prevalence of risk factors according to sex in patients with ACS and LBBB

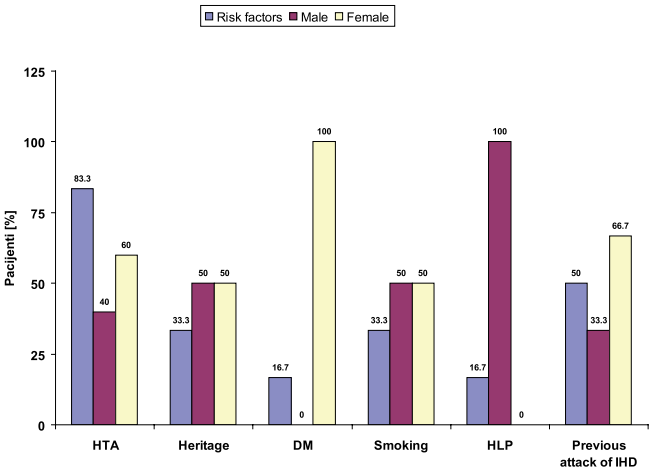

Figure 2. Prevalence of risk factors according to sex in patients without ACS and LBBB

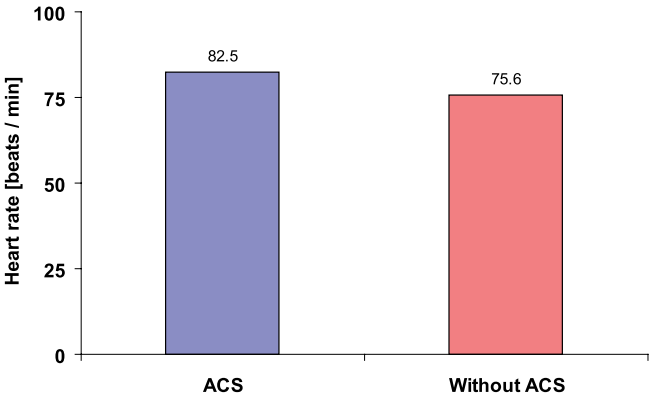

Figure 3. Mean heart rate on admission in patients with LBBB

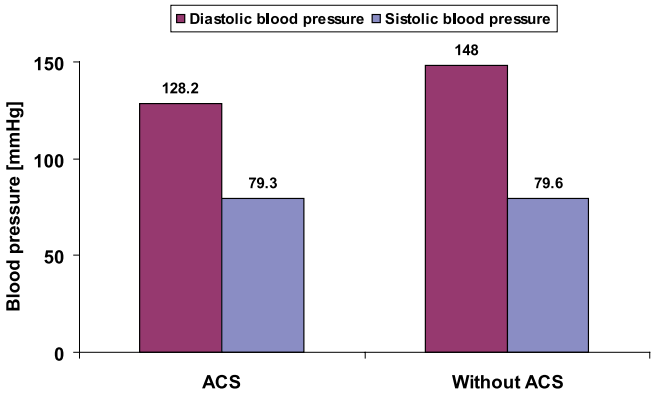

Figure 4. Mean values of systolic and diastolic blood pressure of all patients with LBBB

Table 2. Sensitivity and specificity of expanded Sgarbossa criteria in patients with ACS and LBBB (1, 2).

Expanded Sgarbossa criteria	Sensitivity	Specificity
ST-segment elevation ≥1 mm and concordant with QRS complex	/	/
ST-segment elevation ≥5 mm and disconcordant with QRS complex	/	/
ST-segment depression ≥ 1mm in lead V_2, V_3	/	/
Presence of Q waves in two contiguous precordial leads or in two limb leads	0,14	1
R-wave regression from leads V_1 to V_4	/	/
QS pattern from leads V_1 to V_4 and rS to V_1	0,14	0,66
Positive T waves in lead V_5 or V_6	0,5	0,73
Sign of Cabrera - Notching ≥ 0.05 sec in the ascending limb of the S wave in lead V_3 or V_4	0,5	0,83
Sign of Chapman - Notching ≥0.05 sec in the ascending limb of R waves in lead I, aVL, V_5, or V_6	0,79	0
Terminal S wave in lead V_5 or V_6	0,5	0,83
Left-axis deviation	0,57	0,66

Different groups of medications administered in patients with LBBB and with or without ACS are shown in table 3.

Table 3. Use of medications in patients with LBBB and with or without ACS

Drug	ACS (+)	ACS (-)
Aspirin	92,85%	50%
Clopidogrel	85,71%	0%
Nitrates	100%	50%
Heparin	100%	33,33%
Beta blocker	78,57%	50%
ACE inhibitor	71,43%	50%
Statin	71,43%	16,66%

Intrahospital mortality in patients with LBBB was estimated using the number of patients who survived, 12 (ACS) and 6 (without ACS), and the number of patients

who died during the study, 2 (ACS) and 0 (without ACS) ($p = 1.00$; Fisher's exact test).

DISCUSSION

For the last 50 years, numerous studies have been devoted to the problem of recognizing acute coronary syndrome in the left bundle branch block of the His bundle; however, the most prominent study was performed in 1996 by Elena Sgarbossa. Based on the above mentioned study, Sgarbossa criteria were formulated and have played an indisputable role in establishing the diagnosis of acute coronary syndrome in patients with left bundle branch block of the His bundle. This is based on the fact that early establishment of the diagnosis and application of this therapy with these patients has contributed to better prognosis.

As indicated by the results from previous studies, acute LBBB in ACS occurs in 0.5% - 9% of patients, has a sensitivity of 42%, a specificity of 65%, and is three times more likely to indicate acute ischemia than an old LBBB (3, 9).

According to the results of our study, the presence of acute coronary syndrome of the left bundle branch block of the His bundle in comparison to total number of patients with acute coronary syndrome was 4.2%, which correlates well with research conducted thus far (3). The average age of our patients was 60. According to foreign studies, LBBB occurs in 2.7% of patients with acute coronary syndrome in populations younger than 65 years of age and in 10.5% of the population above 75 years of age (5). Judging by the data from currently available literature, ACS is present in 25% of patients with previous or newly arisen LBBB. It is well known that in the subgroup of patients younger than 65 years of age, chest pain appears in 37% of patients, whereas among the population above 75 years of age, 50% of patients have no chest pain but have ACS (5). Our study did not involve observation of all examined patients with LBBB but only investigated those patients who had LBBB accompanied by chest pain, which was the reason why they were hospitalized at the Coronary unit. Consequently, the incidence of ACS was significantly higher (70%). In our study the leading risk factor in patients with left bundle branch block and acute coronary syndrome was hypertension, immediately followed by heredity and diabetes mellitus, principally in patients of the male sex. The other studies had similar results (8, 17). Fisher's exact test showed the only link between DM and ACS in patients with LBBB ($p = 0.018$). When binary logistic regression was used (with DM, sex, HTA, HLP, and smoking as confounding variables), DM had a statistically significant influence on the occurrence of ACS in LBBB; it increased the likelihood of development of ACS in patients with LBBB by 42 times (odds ratio ŠORĆ 42; 95% CI, 1.176 to 1497.973; $p < 0.05$). Our study indicated that patients with acute coronary syndrome and LBBB had high heart frequency compared to those without ACS. High heart frequency is a prognostic index for cardiovascular risk. The relationship between heart frequency and mortality of coronary and cardiovascular diseases was proved in various studies. The risk of death increased as much as five-fold in men whose heart rate was higher than 88 beats per minute compared to men who had heart frequency range lower than 65 beats per minute (8, 16). The mean heart rate in a group of patients with ACS and LBBB was 82.5 beats per minute while in a group of patients without ACS, the heart rate was 75.6 beats per minute. The difference between those two values had no statistical significance (Student's T-test) ($p = 0.447$) due to the small size of the sample group; however, there was an increase in heart rate in the group with ACS and LBBB. The ranges of systolic blood pressure were lower in patients with acute coronary syndrome due to their antihypertensive treatment while the ranges of diastolic blood pressure were the same in both subgroups of patients. There were no significant differences between mean values of systolic blood pressure ($p = 0.202$) and diastolic blood pressure ($p = 0.981$).

Framingham's study showed that in patients suffering from hypertension, heart frequency range was related to various types of mortality regardless of other risk factors such as mortality for any other reason, due to coronary disease or cardiovascular disease.

Two of our male patients with ACS, with hypertension and heart rate on admission higher than 90 beats per minute died. This result was in concordance with CORDIS (Community Research and Development Information Service) (16) research that has indicated that cardiovascular death is two times higher in patients with heart frequency range above 90 beats per minute (8). In all studies performed up to this point in patients with LBBB, the three most significant criteria, concordant with QRS complex ST segment elevation $\geq$ 1mm, disconcordant with QRS complex ST segment elevation $\geq$ 5mm, and depression of ST segment in the leads V_2, V_3 $\square$ 1mm, were observed along with the additional criteria that, when associated, could point to acute coronary syndrome in certain number of cases (1, 2).

ST elevation > 1mm concordant with QRS complex has a sensitivity of 73%, which signifies that out of 100 patients with ACS and LBBB, 73 of them have this EKG change whereas 27 of them do not possess this EKG change. A specificity of 92% means that out of 100 individuals with LBBB and without ACS, eight of them had this change (false-positive), while 92 of them did not have this change present.

In our study group of 20 patients, there were 14 patients with elevated levels of cardio-specific enzyme, which served as a confirmation for ACS. We have shown that the expanded Sgarbossa criteria should not be neglected when establishing a diagnosis of ACS, particu-

larly when the following criteria are present: negative T wave in the leads V_5, V_6, a sign of Cabrera- ascending limb of the S wave in V_3 or V_4, the presence of an S wave in lead V5 or V6, the presence of a Q wave in two contiguous precordial leads, and left-axis deviation (Table 2).

Due to differences between practical recommendations from the AHA (American Heart Association, 2004) (19) and ECS (European Heart Association of Cardiology, 2008) (20) thrombolytic therapy has become justified in all patients with chest pain and (probably) de novo LBBB (7, 12). Consequently, our patients without presence of Sgarbossa criteria (1-3) did not receive thrombolytic therapy.

However, there are studies that suggest that the administration of thrombolytic therapy in patients who have LBBB and are older than 75 years is less successful (three vessel disease is more common) and related to side effects of thrombolytic treatment (stroke, haemorrhage, myocardial rupture) (10, 14, 15).

Administration of often drugs in patients with ACS (acetic acid, clopidogrel, heparin, nitrates, beta blockers, ACE inhibitors and statins) was considerably higher than in the foreign studies (Table 3) (5).

The occurrence of LBBB in AMI is related to increased mortality during hospitalization and one month and one year after hospitalization; however, if the left ventricular ejection fraction (LVEF) and comorbidity are taken into account, the difference in mortality between patients with and without LBBB disappears (4, 9), a finding that has been shown in an investigation by Stenestrand et al. (9).

In our investigations we have shown that intrahospital mortality is higher if ACS is associated with LBBB; however, Fisher's exact test showed no significant dependence between ACS and mortality ($p = 1.00$).

It is well known that intrahospital mortality increases with severity of heart failure (4). LBBB is more common in older male patients and is very often connected to anterior wall myocardial infarction with the presence of heart failure and comorbidity. Therefore, the mortality of these patients was found to be as high as 22% (5) or to range from 18% to 27% depending on whether chest pain was present or not (6).

None of our patient had 1-3 Sgarbossa criteria for ACS and anterior wall myocardial infarction, thus there was no difference in intrahospital mortality between patients with and without ACS and LBBB.

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HERPES ZOSTER IN A PATIENT WITH RHEUMATOID ARTHRITIS AND MIXED CONNECTIVE TISSUE DISEASE

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HERPES ZOSTER KOD PACIJENATA SA REUMATOIDNIM ARTRITISOM I MEŠOVITOM BOLEŠĆU VEZIVNOG TKIVA

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ABSTRACT

Herpes zoster (HZ) is a viral disease caused by the Varicella-Zoster virus. Primary infections usually occur in childhood when the virus causes a varicella infection. Virus is then detained in spinal nerve ganglia in latent form. In adults, triggers such as stress, trauma, chronic and malignant diseases, AIDS, and even sunburns, lead to reactivation of the virus and clinical manifestation of the herpes zoster infection. The incidence of herpes zoster is higher in elderly populations and in people with damaged cell-mediated immunity. The typical clinical appearance of herpes zoster comprises clusters of clear vesicles and blisters on the erythematous base; clusters are unilateral and localized in one dermatome, which is innervated by the affected spinal nerve ganglion. In immunocompromised patients, the clinical appearance of HZ can be varied, often accompanied by local and/or systemic complications that, in some cases, can lead to lethal outcomes. We present a case of herpes zoster infection in a 59-year old woman with a two-year history of rheumatoid arthritis (RA) and mixed connective tissue disease (MCTD). The illness exhibited acutely beginning with fever and malaise. The ulcerative form of herpes zoster was clinically present, with slow and only slightly archived epithelialisation. As a result, an atrophic scar remained on the affected skin.

Key words: Herpes zoster, rheumatoid arthritis, mixed connective tissue disease.

ABSTRAKT

Herpes zoster (HZ) je virusno oboljenje koje uzrokuje Varicella-Zoster virus. Primarna infekcija se obično javlja u detinjstvu kada virus uzrokuje varicellu. Posle toga, virus se zadržava u ganglionima spinalnih nerava u latentnom stanju. Kod odraslih, trigeri kao stres, trauma, hronične i maligne bolesti, AIDS, ali čak i opekotine od sunca dovode do reaktivacije virusa i kliničke manifestacije herpes zostera. Incidenca herpes zostera je viša kod odraslih i osoba sa oštećenim ćelijskim imunitetom. Tipičnu kliničku sliku herpes zostera čine grupisane vezikule i/ili bule ispunjene bistrom, seroznom tečnošću na eritematoznoj osnovi, lokalizovane unilateralno, u dermatomu koji inerviše zahvaćeni ganglion spinalnog nerva. Kod imunokompromitovanih bolesnika klinički izgled HZ može biti različit, često udružen sa lokalnim i/ili sistemskim komplikacijama koje, u nekim slučajevima, mogu dovesti do letalnog ishoda. Prikazujemo slučaj herpes zostera kod 59 godina stare žene koja dve godine boluje od reumatoidnog artritisa i mešovite bolesti vezivnog tkiva. Bolest je imala akutan početak praćen visokom temperaturom i malaksalošću, ulcerativna forma herpes zostera je bila klinički prisutna, zarastanje sporo i teško dostignuto. Kao rezultat, atrofični ožiljak je ostao na zahvaćenoj koži.

Ključne reči: herpes zoster, reumatoidni artritis, mešovita bolest vezivnog tkiva.

INTRODUCTION

Herpes Zoster is an infectious disease caused by the Varicella-Zoster virus (family Herpetoviridae), and it usually occurs in adults. The illness is the second manifestation of the Varicella-Zoster infection. The first attack of the virus happens in most cases during childhood, when it causes varicella infection (1). After varicella infection, the virus remains in spinal nerve ganglia in a latent form (2). In some cases, when the immune system is disturbed by benign causes (stress, trauma, sunburns, etc.) or severe causes (chronic and malignant diseases, AIDS, radiotherapy, etc.), the virus starts to replicate and becomes active again after many years in latency. Clinical manifestations of herpes zoster are usually unilateral (in severe immunodeficiency, symptoms can be disseminate and clinically resemble varicella) and located in one dermatome, which is innervated by the affected spinal nerve. In the beginning, the skin is sensitive; pain is present and can be so severe as to mimic some other diseases, such as heart attack, migraine, or acute appendicitis (1). After 1-2 days, skin lesions can be seen.

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On the erythematous base, varying numbers of vesicles and blisters with serous or serohemorrhagic fluid are present. They leave deep erosions or ulcers that can lead to scarring. The most common problem following herpes zoster infection is hyperpigmentations. Pain is present at the very beginning of the illness, during, and 2-3 months (sometimes longer) after the resolution of skin manifestations (postherpetic neuralgia) (1). A diagnosis is usually made on clinical grounds, but histopathology, viral culture, and serological examination can also be used to confirm the diagnosis. Antiviral drugs (Acyclovir, Famacyclovir, Valacyclovir) and antiviral ointments are the basis for herpes zoster therapy. Antibiotics are also used, as well as vitamins, analgesics, and sedatives (in difficult cases of postherpetic neuralgia) (1). In immunosuppressed patients, the clinical manifestation of herpes zoster is complicated. Ulcerative, necrotic or disseminate forms of herpes zoster are seen in these patients; after resolution, they leave varicelliform, deep scars on the affected skin. In cases like this, relapses of the disease are possible, but they are very rare.

CASE REPORT

A 59-year old female patient with a two-year history of rheumatoid arthritis (RA) and mixed connective tissue disease (MCTD) was admitted to the Dermatology Unit with clinical manifestations of herpes zoster (HZ) infection localized on the right half of the trunk in the dermatome that innervates TH 12. Subjective, severe pain was present, as well as fever and malaise. Clinical examination showed numerous vesicles and bullas with hemorrhagic fluid on the erythematous base (figure 1). After 48 hours, these efflorescences became confluent and the affected epidermis became eroded, leaving large, deep, erosive surfaces in some parts, covered with eschara (figure 2). A diagnosis was made on clinical grounds. Laboratory studies showed a normal sedimentation rate; a complete blood count and urine analysis were within normal limits. Rheumatoid factor was elevated. Abdominal ultrasound and chest radiography showed no abnormalities. Antiviral therapy (Acyclovir tbl 400 mg, administered 5 times a day for 10 days) was used to treat herpes zoster; antibiotic therapy (Cephtriaxon amp 2 g/day for 7 days) was used as well. Topical therapy comprised an erosive surface antibiotic gel and local antiseptic. To treat RA and MTCD, the patient was given Methotrexate (15 mg per week) and Prednisolone (15 mg per day). The reaction to antiviral and antibiotic therapy was not very good; the clinical course of a viral disease that usually lasts for 2 to 3 weeks was delayed. Epithelialisation of the deeply eroded surface was very slow (figure 3). It took two months until a large, atrophic scar appeared on the skin affected by infection. Severe pain was present throughout the entire course of the illness, and postherpetic neuralgia lasted for 3 months. Local and oral analgesics were used to suppress the pain.

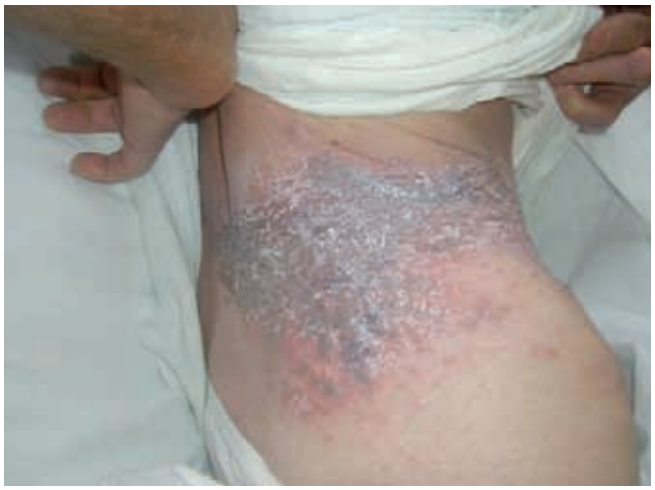

Figure1. Numerous vesicles and bullas with hemorrhagic fluid covered with anesthetic talc, localized on the erythematous base on the right side of the trunk.

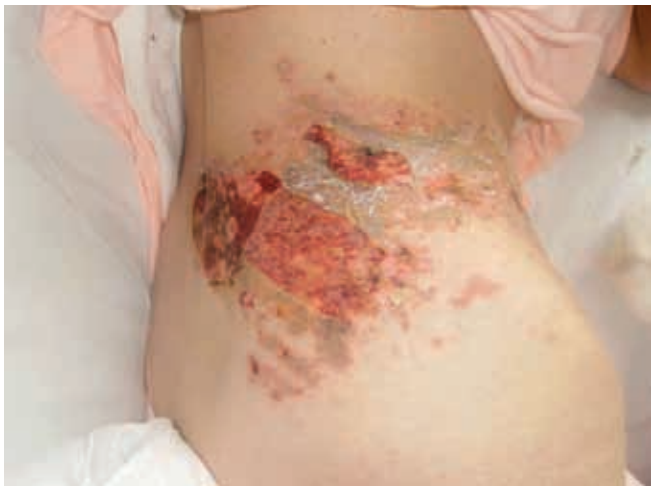

Figure 2. Ulceration covered with eschara and pieces of eroded epidermis.

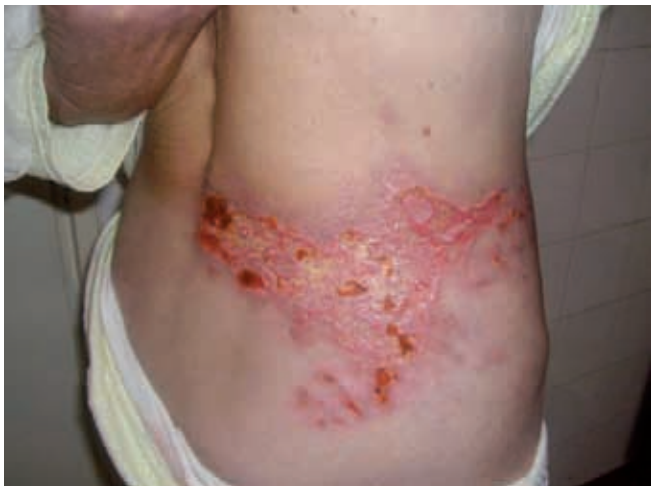

Figure 2. Ulceration covered with eschara and pieces of eroded epidermis.

DISCUSSION

HZ occurs more frequently in patients with RA and MTCD than in the general population (3). Impaired cellular immunity is probably the reason for this high incidence (4). Clinical manifestation of HZ in patients with RA and MCTD varies. Some authors suggest that the course of HZ in these patients is benign and self-limiting, while other authors have observed patients challenged with life-threatening herpes zoster infection, in which case skin manifestations were accompanied by severe systemic involvement (3,5,6). HZ causes significant morbidity in immunosuppressed patients, while the reported mortality rate is below 5%. Complications are much more frequent in these patients compared to the general population, including meningoencephalitis, hepatitis, retinopathy, vasculopathy, coetaneous dissemination, and particularly delayed healing (7). Our experience supports findings by Antonelli et al (3). In our case, the disease had an acute onset with subjective features such as fever, malaise and severe pain. The ulcerative type of HZ was presented on the skin without any systemic manifestation. Despite a good recovery, the course of the disease was prolonged and self-limiting because it took two months until epithelialisation was complete.

ABBREVIATIONS:

HZ- herpes zoster
MCTD- mixed connective tissue disease
RA- rheumatoid arthritis

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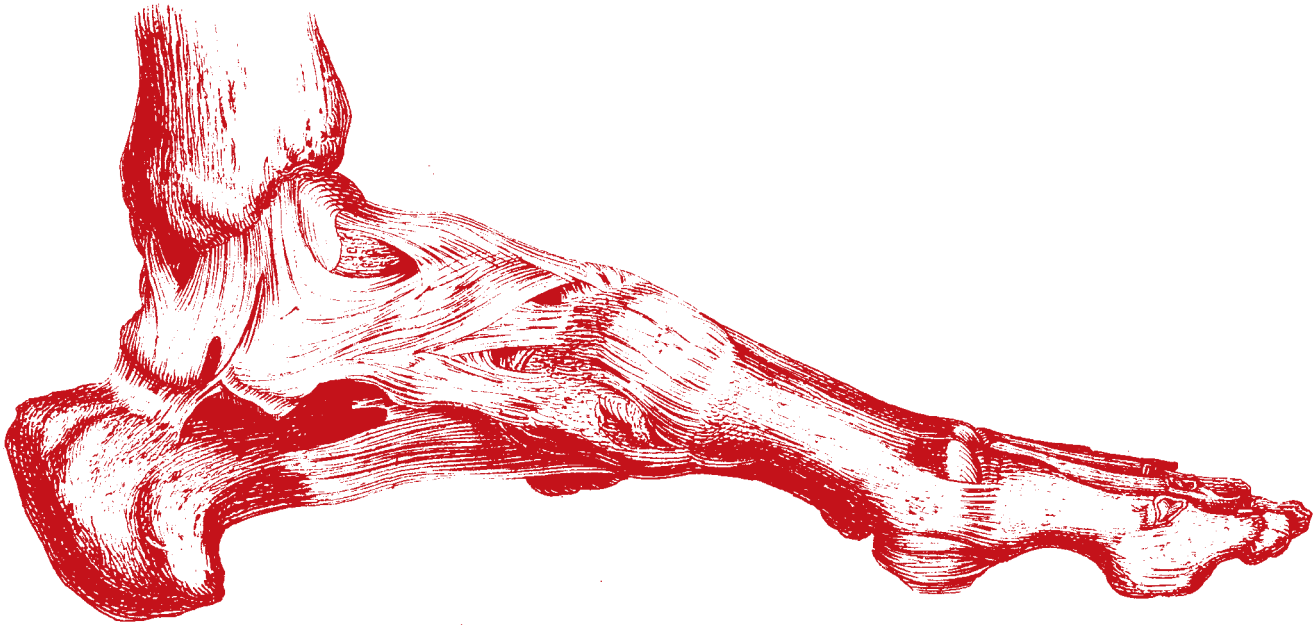

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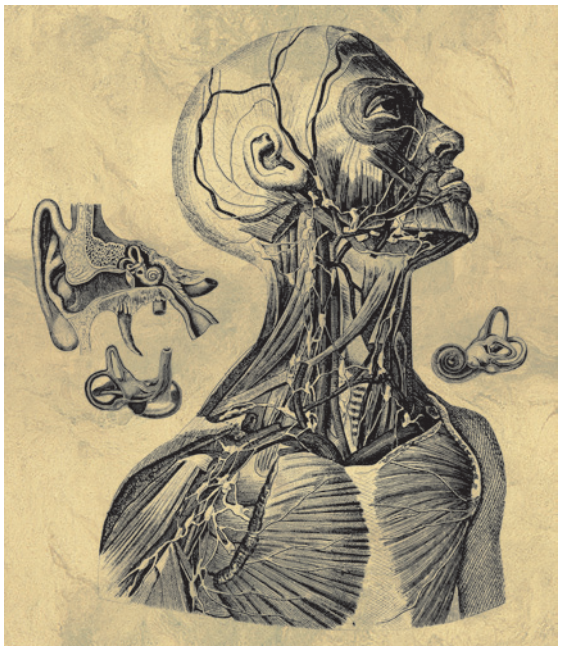

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