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POSITRON EMISSION TOMOGRAPHY - INTRODUCTION INTO THE PUBLIC HEALTH CARE SYSTEM OF SERBIA

Vladimir Obradovic

Positron emission tomography (PET) is an imaging modality within the nuclear medicine field and is capable of making an *in vivo* "measurement" of physiological and biochemical processes at the cellular and molecular levels.

The technique uses a biologically active substance labelled with a positron-emitting radioactive isotope (e.g., fluorine-18 ^{18}F , carbon-11 ^{11}C , nitrogen-13 ^{13}N , oxygen-15 ^{15}O), with ^{18}F and ^{11}C being the most commonly used. The radiolabeled molecules, named radiopharmaceuticals, maintain their properties after administration in the patient and are metabolised through specific cellular processes. Due to the differing metabolic processes between diseased and healthy tissues and organs, detected radiation, either from a whole body or from targeted scan with a PET scanner, will enable visualisation and localisation of the studied disease or disorder. The possibility of using the acquired PET data, along with other modalities and computer processing, for additional quantitative assessment considerably enhances PET's diagnostic potential. More than 120 PET radiopharmaceuticals are available and used for clinical and research purposes. Because the half-life of the most commonly used positron emitters, except for ^{18}F , is usually only minutes long, they must be produced on-site where the PET scanner is located. Therefore in addition to the PET scanner, contemporary centres are equipped with a medical cyclotron, for the production of positron emitters, and an automated radiochemistry laboratory, for the synthesis of different radiopharmaceuticals. Hybrid PET/CT scanners are more frequently used than stand-alone PET scanners so that functional changes visualised with PET can be simultaneously localised with fused CT images. Hybrid SPECT/PET/CT and PET/MRI devices are also being developed.

Owing to its exceptional potential, PET has a major role in modern diagnostics of the most severe and frequent diseases, and contemporary tertiary health care institutions will not maintain their rank if they are not equipped with PET technology. PET is most frequently used in oncology. The metabolic processes in tumour tissues are accelerated

and enhanced, so with the appropriate radiopharmaceuticals, the viable tumour tissue will be clearly imaged. The technique is highly sensitive for detection of local and distant metastases and differentiation between resting tumours, recurrent viable tumours, and fibrosis caused by surgical or radiation treatment. Moreover, PET is used to assess tumour malignancy, disease prognosis, and tumour sensitivity to specific chemotherapy agents. PET also improves the precise planning of radiation therapy. For this reason, introducing PET into the routine oncologic diagnosis is of the utmost importance.

Through the development of radiopharmaceuticals, it is also possible to visualise different aspects of brain function using PET, so the modality's applications are increasing for diagnosis in neurology, especially for different types of dementia, early diagnosis of parkinsonism, and precisely determining the epileptic foci for surgical removal.

As for cardiology, PET enables precise viability assessment of the heart muscle in patients with coronary disease that significantly contributes to the decision-making process in contemporary methods of interventional cardiology, coronary blood vessel bypass, and heart transplantation. Due to the rapid and accurate diagnosis, PET facilitates timely and appropriate treatment that can lead to better prognosis of the disease. Simultaneously, the patients are spared long and frequently invasive diagnostic and therapeutic procedures. All of the above-mentioned factors contribute to significant savings in the health care budget.

Establishing the PET Centre in the Clinical Centre of Serbia was first proposed three decades ago (Academician Vladimir Bošnjaković). In 1998, the idea of introducing PET into the domestic public health care system, which included the National PET Centre as its basis, was presented (Prof. Dr. Vladimir Obradović) and officially approved by the Yugoslav Society of Nuclear Medicine. In accordance with that concept, the National PET Centre is considered to be the basic clinical, educational, and scientific PET in-



stitution in Serbia. Following its completion, establishing smaller (“satellite”) PET centres has also been planned at the Institute for Oncology in Sremska Kamenica, the Clinical Centre of Niš, the Clinical Centre of Kragujevac, and the Military Medical Academy in Belgrade.

As early as 2005, the Minister of Health appointed the Expert Team, which consisted of distinguished domestic experts, including five members of the Serbian Academy of Science and Arts, which has been responsible for bringing the National PET Centre in the Clinical Centre of Serbia to fruition. The Team adopted the plan detailed above. The decision was also made for the parts of PET Centre to be independently constructed and equipped; the Clinical Department required considerably less time than the Department for production of positron radiopharmaceuticals. At the time, there was such a great need for the diagnostic PET applications to be available that ^{18}F FDG (^{18}F -deoxyglucose) needed for PET was going to be temporarily imported until the cyclotron and radiopharmaceutical facility could be built.

The Clinical Department of the National PET Centre was supplied in turnkey condition by Siemens Healthcare. The space for the PET Centre comprised about 700 square meters in the basement of the Polyclinic Building of the Clinical Centre of Serbia. Nine supply companies, which Siemens had contracted to provide a range of services from radiation protection to computer equipment and furnishings, constructed and equipped the Centre with the necessary facilities. Although the premises and infrastructure had been constructed for two PET/CT systems, currently only one has been installed. Another PET/CT (or PET/MR) device should be installed together with the medical cyclotron and radiochemical laboratory in the project’s final phase. During 2006, through a technical cooperation project with International Atomic Energy Agency (IAEA), three physicians trained abroad for several months in PET centres. A new project with the IAEA has been under way, and nine Centre members of a multidisciplinary team will

also complete the PET training by the end of 2010. Due to some administrative and technical issues, construction of the Clinical Department was delayed, and the Department was not provided with the PET/CT scanner before its completion, in accordance with all necessary safety and other standards, nor was the basic medical team educated in PET applications. The Clinical Department of the National PET Centre was completed in September and officially opened on October 22, 2009. A different approach was taken for the Institute for Oncology in Sremska Kamenica: it was provided with a PET/CT by the end of 2007 (while the facilities were still under construction) and started routine weekly PET/CT applications at the beginning of 2009. During that time, the hybrid device was primarily used only for CT applications. The first diagnostic PET applications in Serbia were performed at this institute.

According to the plan adopted by the Ministry of Health’s Expert Team, the next step will be construction of the Department for Production of Positron Radiopharmaceuticals as a part of the National PET Centre, including installation of a medical cyclotron and radiochemistry laboratory. Then, the Centre will be able to acquire PET images with radiopharmaceuticals labelled not only with ^{18}F but also with the short lived positron emitters ^{11}C and ^{13}N .

Finally, the National PET Centre will be the country’s main provider for molecular imaging and able to fulfill all of its important roles: significantly improve diagnostic work-up of the most serious and frequent (oncologic, neurologic, and cardiologic) diseases, minimise the necessity to perform costly surgical and other therapeutic procedures, train medical doctors and others interested in PET, investigate in vivo at cellular and molecular levels, and supply the future “satellite” domestic PET centres with ^{18}F FDG and other radiopharmaceuticals labelled with ^{18}F .

Prof. Dr. Vladimir Obradović,
President of the Expert Team
of the Ministry of Health for PET

A NEW SE MI-QUANTITATIVE METHOD FOR DETERMINING LIVER DAMAGE AFTER CONCAVALIN A ADMINISTRATION

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NOVI SEMI-KVANTITATIVNI METOD ZA ODREĐIVANJE STEPENA OŠTEĆENJA JETRE NAKON PRIMENE KONKAVALINA A

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ABSTRACT

Concanavalin A (Con A)-mediated hepatitis is a mouse model of liver injury that resembles autoimmune and viral hepatitis in humans. Because of the similarities in pathogenesis, clinical symptoms and histological characteristics, Con A-induced liver injury is a useful animal model for researching hepatocellular damage in murine and human hepatitis.

Although many experiments have been conducted with the aim to investigate the mechanism of Con A-induced liver injury, a precise method for determining liver damage after Con A treatment has not been established yet.

To improve the study of hepatitis, we established a new semi-quantitative method to determine liver damage after Con A injection using histological examination. Briefly, liver sections were fixed in 10% formalin, embedded in paraffin, and cut into 4- μ m-thick sections. The sections were stained with haematoxylin-eosin and examined under light microscopy (100 \times) to evaluate liver damage. Necrosis of hepatocytes was characterised by standard morphologic criteria (loss of architecture, vacuolisation, karyolysis), and the extent of necrosis was semi-quantitatively determined using digital camera images and the "polyline" tool of Autodesk AutoCAD 2009 software. A detailed procedure for the semi-quantitative determination of liver injury after Con A injection is presented in this paper.

Using this method, the whole tissue section can be analysed. This is a significant advantage compared to similar, previously published methods that analyse randomly chosen microscopic fields. Another big advantage of this method is its simplicity and the availability of the Autodesk AutoCAD 2009 software for public use.

Key words:

Concanavalin A, hepatitis, semi-quantitative method

SAŽETAK

Konkanavalinom A izazvan hepatitis predstavlja mišji model za proučavanje autoimunskog i virusnog hepatitisa ljudi. Konkanavalinom A izazvano oštećenje jetre je relevantan model za proučavanje mehanizama i stepena oštećenja jetre, usled sličnosti u patogenezi, kliničkoj slici i patologiji sa hepatitisom ljudi.

Iako je uradjeno mnogo eksperimenata sa ciljem da se ispita patogeneza Konkanavalinom A indukovano oštećenja jetre, i dalje ne postoji tačno opisan i precizan metod za izračunavanje stepena oštećenja jetre u ovom eksperimentalnom modelu.

Mi smo postavili novi metod za semi-kvantitativno određivanje stepena oštećenja jetre nakon aplikovanja Konkanavalina A i taj metod predstavljamo u ovom radu.

Za histološko ispitivanje, jetre se fiksiraju u 10% formalinu, nakon čega se prave preparati debljine 4- μ m. Preparati se, nakon bojenja hematoksilin-eozinom, posmatraju svetlosnim mikroskopom (uvećanje 100 \times), fotografišu digitalnim aparatom i korišćenjem „polyline” opcije programa Autodesk AutoCAD 2009 označavaju se polja nekroze hepatocita koje karakteriše gubitak morfologije, vakuolizacija i karioliza. Postupak semi-kvantitativnog određivanja jetre je detaljno opisan u radu.

Korišćenjem ovog metoda, određuje se stepen nekroze u celoj jetri, što je glavna prednost u odnosu na do sada opisane metode kojima se određivao stepen nekroze hepatocita u nasumično odabranim poljima preparata. Takođe, prednost ovog metoda je što se koristi program Autodesk AutoCAD 2009 koji je jednostavan za rad i dostupan na tržištu.

Ključne reči:

Konkanavalin A, hepatitis, semi-kvantitativni metod.

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INTRODUCTION

Viral hepatitis is a serious health problem worldwide, as more than two billion people have been infected by hepatitis B virus, and 170 million people have been infected by hepatitis C virus [1]. An immune-mediated mechanism is responsible for the destruction of virus-infected hepatocytes in human viral hepatitis [2-3]. Due to the highly sophisticated morphological organisation of the liver and the integrity of metabolic pathways and their specific regulation in liver cells, the development of hepatic injury has not been easily studied in cellular systems for many years. A break-through in liver injury research field was made in 1992 when a mouse model of immune-mediated liver injury, which resembles autoimmune and viral hepatitis in humans, was established by intravenous injection of Concanavalin A (Con A) [4-5]. Con A is a mitogenic plant lectin that induces polyclonal T cell activation *in vitro*, and causes, after intravenous injection (in dose >1.5mg/kg), severe and acute liver injury in mice, resulting in clinical and histological symptoms of acute hepatitis within 24 h [4]. Con A strongly binds to hepatocytes' plasma membranes, and hepatocytes can be sensitised or even killed by Con A if it is injected at high concentrations [6]. Although the precise mechanisms involved in the pathogenesis of Con A-induced liver injury are not fully understood, there is direct evidence that the activation of T cells, macrophages, neutrophils and natural killer T cells (NKT) is essential for Con A-induced hepatic injury [4, 6-9]. Liver injury is associated with massive CD4+ T, NKT and macrophage activation, followed by secretion of the following pro-inflammatory cytokines: tumour necrosis factor alpha (TNF- α), interleukin 1 (IL-1), interferon- γ (IFN- γ), interleukin 2 (IL-2), interleukin 6 (IL-6) and granulocyte macrophage-colony stimulating factor (GM-CSF) [10-11]. Marked elevation of transaminases (alanine aminotransaminase (ALT) and aspartate aminotransferase (AST)) in mouse blood occurred after Con A injection, with the maximum levels found between 6 and 8 hours after administration [4-5, 12]. In addition, intravenous injection of Con A resulted in a remarkable disruption of mice liver tissue, manifested by widespread areas of necrosis and inflammation within the liver lobules [4-5, 13].

Because of the similarities in pathogenesis, clinical symptoms and histological characteristics, Con A-induced liver injury is a readily available and useful animal model relevant for the research of hepatocellular damage in murine and human hepatitis [4-5].

MATERIALS AND METHODS

Animals

To determine Con A-induced liver injury, we used 6-8 weeks old male BALB/c mice. Mice received standard laboratory chow and water *ad libitum* and were kept in 12 h light/dark cycles.

Con A-induced liver injury

Con A was purchased from Sigma Chemical Co. (St. Louis, MO).

Mouse liver damage was induced by injection of Con A (12 mg/kg), dissolved in 250 μ L of saline, through the tail vein.

Determination of liver injury

Transaminase (ALT and AST) measurement and liver histology are the standard methods for determining liver damage. To measure the levels of AST and ALT, plasma samples or sera were collected from mice at indicated time points after Con A injection. AST and ALT levels were determined by a biochemical kit (Olympus medical kit) according to the manufacturer's instruction.

Compared with transaminase measurement, which is a standardised procedure, there is no standardised and generally accepted semi-quantitative method for determining hepatocellular damage. Because of that, we present here, a new method for quantifying hepatic injury after intravenous administration of Con A using histological examination.

For histological examinations, livers were fixed in 10% formalin, embedded in paraffin, and cut into 4- μ m-thick sections. The sections were stained with haematoxylin-eosin and examined under light microscopy to evaluate liver damage.

Necrosis was examined using low-power (100 \times) light microscopy, and images were obtained using a digital camera. The area of necrosis was quantified using the Autodesk AutoCAD 2009 software application for design and drafting.

Liver tissue sections were photographed using (100 \times) light microscopy and a digital camera. Each photo of the tissue sample was imported into a newly-created Autodesk AutoCAD 2009.dwg file. Using the "polyline" tool, we drew "polyline" regions around the whole sample (marked with A) and around each of the necrotic areas in the photo (marked with B). We then determined the surface area of the drawn regions. First, we determined the surface area of the A region, and then we determined the surface areas of each of the B regions (one by one). The surface area of each drawn region is presented as a unitless number in the Autodesk AutoCAD program. After we examined all of the photos from a whole liver tissue section, we calculated the percentage of necrotic area using the formula:

$$N = Bt \times 100 / At$$

where:

N is the percentage (%) of necrotic area in the whole tissue section,

At (A total) is the sum of the sample surface areas in the whole tissue section ($At = A1 + A2 + \dots + An$, where n is the number of photos),

Bt (B total) is the sum of the necrotic surface areas in the whole tissue section ($Bt = B1 + B2 + \dots + Bm$, where m is the number of marked necrotic fields).

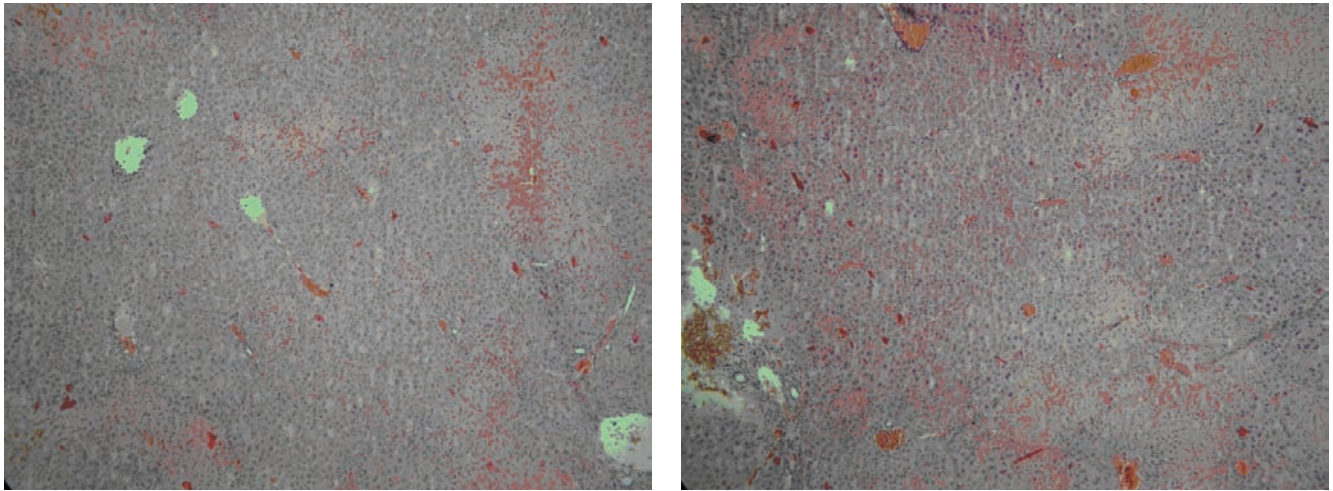


Figure 1 and 2. Mice livers 24 hours after intravenous administration of Con A.

RESULTS

Histological analysis of the liver sections of Con A-treated mice showed widespread areas of necrosis and inflammation within liver lobules and around central veins and portal tracts. Extensive lesions are characterised by massive hepatocytes, coagulative necrosis, and cytoplasmic swelling of most living hepatocytes (Figures 1 and 2). Nuclear chromatin condensation was found frequently, which indicates hepatocyte apoptosis. Moderate infiltration of lymphocytes and mononuclear cells in portal areas and around central veins was also observed.

The necrosis of hepatocytes was characterised by standard morphologic criteria (loss of architecture, vacuolisation, karyolysis, increased eosinophilia), and the extent of necrosis was semi-quantitatively estimated using the method we presented here (Figures 3 and 4).

Using this semi-quantitative method, hepatic necrosis was assessed in each section as the percentage of the liver parenchyma with necrotic damage.

Further, the extent of liver damage was scored with a grade from 0 to 4 as follows:

Grade 0: normal histology where necrotic area is 0%;

Grade 1: minor necrosis, necrotic area covered < 10% of the whole tissue section;

Grade 2: necrotic area covered 10–25% of the whole tissue section;

Grade 3: necrotic area covered 25–50% of the whole tissue section;

Grade 4: necrotic area covered >50% of the whole tissue section.

DISCUSSION

This method is a newly designed, reliable semi-quantitative way to determine liver damage in Con A-induced hepatitis.

Using this method, the whole tissue section is analysed. This is a significant advantage compared with similar, previously published methods that analysed randomly chosen microscopic fields [11, 14].

In addition, the big advantage of this method is its simplicity and the availability of Autodesk AutoCAD 2009 software for public use.

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Figure 3 and 4.

The whole sample (marked with an A) and necrotic areas (marked with a B) were labelled with the “polyline” tool (Autodesk AutoCAD 2009 software).



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ASSESSMENT OF MYOCARDIAL VIABILITY WITH DOBUTAMINE STRESS ECHOCARDIOGRAPHY IN PATIENTS WITH LOW EJECTION FRACTIONS AND DIABETES MELLITUS TYPE II

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EHOKARDIOGRAFSKA PROCENA VITALNOG MIOKARDA DOBUTAMIN STRES ECHOKARDIOGRAFIJOM KOD PACIJENATA SA NISKOM EJEKCIJOM I DIJABETES MELLITUSOM

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Introduction

The prediction of improvements in left ventricular ejection fraction (EF) after revascularisation in patients with ischemic cardiomyopathy relies only on the extent of viable myocardium. The amounts of viable tissue and scar tissue are important but their relationship is different in diabetic and non-diabetic patients.

Design and Methods:

This study included 50 patients with a low EF (EF<40% by the Simsons method) divided into two groups. The first group consisted of 30 patients with registered coronary artery disease and normal glycoregulation, and the second group consisted of 20 patients with diabetes mellitus and registered coronary artery disease. All patients underwent dobutamine stress echocardiography before surgical revascularisation and 8 weeks after surgery (2-5 months). Dobutamine infusion was terminated at 15 µg/kg/min.

Results:

The mean number of hypokinetic segments was 4.32±2.9 before testing, 1.9±2.07 at a 15 µg/kg/min dose of dobutamine, 2.5±2.12 after revascularisation in the group with diabetes mellitus type II and 4.77±2.11, 1.87±2.18, and 2.97±2.28, respectively, in the group without diabetes mellitus type II. The mean number of akinetic segments was 5.95±2.63, 5.45±2.65 and 5.35±2.62 in the group with diabetes mellitus type II and 4.57±1.68, 3.5±2.26, 3.2±2.16 in the group without diabetes mellitus type II. The wall motion score index (WMSI) was 1.99±0.32 before and 1.86±0.31 after revascularisation in the first group and 1.85±0.27 and 1.58±0.24, respectively, in the second group. The sensitivity for the detection of viable myocardium was 100% CI (93%-100%) in both groups, and the specificity was 96% CI (93%-98%) in the group with diabetes mellitus type II and 91% (89%-95%) in the group without diabetes mellitus type II.

Conclusions:

Our study shows that recovery of function occurs in a sizeable number of revascularised dysfunctional segments. This method was very helpful for the assessment of truly "viable" segments in patients with a poor prognosis.

Key words:

dobutamine, echocardiography, diabetes mellitus

Uvod:

Poboljšanje ejection frakcije leve komore nakon revascularizacije miokarda kod bolesnika sa ishemijskom kardiomiopatijom zavisi od količine vitalnog (vijabilnog) miokarda. Odnos između količine vitalnog i ožiljnog tkiva je različit u odnosu na udruženo prisustvo šećerne bolesti.

Materijal i metode:

U studiju je uključeno 50 pacijenata sa niskom ejection frakcijom (EF<40%-Simsonovom metodom), prva grupa od 20 pacijenata bila je sa dijabetes mellitusom tip II i dokazanom koronarnom bolešću, dok je druga grupa od 30 pacijenata bila bez prisustva dijabetes melitusa, a sa dokazanom koronarnom bolešću. Svi pacijenti su podvrgnuti dobutamin stres ehokardiografskom testu pre i 8 nedelja nakon revascularizacije. Infuzija dobutaminom je prekinuta na III stadijumu pri koncentraciji od 15µg/kg/min.

Resultati:

Prosečan broj hipokinetičnih segmenata bio je 4,32±2,9 pre testa, 1,9±2,07 na 15µg/kg/min dozi dobutamina i 2,5±2,12 nakon revascularizacije u grupi sa dijabetesom, odnosno 4,77±2,11; 1,87±2,18 i 2,97±2,28 u grupi bez dijabetesa. Srednji broj akinetičnih segmenata iznosio je 5,95±2,63; 5,45±2,65; 5,35±2,62 u različitim stadijumima testa, kao i nakon revascularizacije u grupi sa dijabetesom i 4,57±1,68; 3,5±2,26; 3,2±2,16 u grupi bez dijabetesa. Indeks skora zidne pokretljivosti iznosio je 1,99±0,32 pre i 1,86±0,31 nakon revascularizacije u prvoj i 1,85±0,27; 1,58±0,24 u drugoj grupi. Senzitivnost metode za detekciju vijabilnog miokarda iznosila je 100% CI(93%-100%) u obe ispitivane grupe, dok je specifičnost bila 96% CI(93%-98%) u grupi sa dijabetesom i 91%(89%-95%) u grupi bez dijabetes melitusa.

Zaključci:

Naša studija je pokazala da se poboljšanje funkcije nakon revascularizacije javilo u značajnom broju disfunkcionalnih segmenata leve komore. Ova metoda se čini veoma korisnom u proceni „stvarno živih“ segmenata kod pacijenta sa lošom prognozom.

Ključne reči:

dobutamin, ehokardiografija, dijabetes melitus

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Myocardial viability is related to a deterioration in contractile function that is potentially reversible if adequate restoration of coronary circulation occurs. Recent findings have indicated that the degree of dysfunction is the main parameter influencing long-term survival in these patients (1, 2). Left ventricular (LV) dysfunction is not necessarily irreversible and can be improved after myocardial revascularisation. Patients with expressive dysfunction of the LV can gain the greatest benefit from bypass surgery because they represent the most sensitive group for the progressive loss of the contractile myocardium; however they also have the highest mortality rate when it comes to surgical procedures. When surgical revascularisation of the myocardium is discussed, it is necessary to take into account its adequacy, graft occlusion, restenosis, and the existence of associated myocardial disease, such as LV hypertrophy. Clinicians primarily treat patients, not myocardial segments, and recent studies have taken into account global LV function, physical capacity, quality of living and mortality as the most important parameters (3). Other factors that are taken into account while defining diagnostic tests are feasibility, accessibility and the experience of the test performer. Regional myocardial dysfunction after myocardial infarction is not always manifested by irreversible damage to the entire affected tissue. Shortly after an acute ischemic event, stunned myocardium spontaneously disappears.

In 1990, stress echocardiography was used for the first time to detect vital myocardial tissue by application of small doses of dobutamine, a drug that recovers the entropic reserves, by Pierard (4). Today, this method is the gold standard due to its relatively high sensitivity, specificity and diagnostic accuracy, with a low cost of performance compared to other diagnostic techniques. The application of pharmacological echocardiography dobutamine stress testing in the most difficult group of patients with low ejection fractions, as well as the disclosure of vital tissue in diabetic patients indicated for surgical and percutaneous myocardial revascularisation is of the highest importance, primarily due to its direct impact on the outcome and prognosis of future procedures.

As the literature does not provide sufficient data on either the evaluation of the vital myocardium in coronary patients with long-term diabetes mellitus type 2 or the results after myocardial revascularisation, it is important to investigate this problem, which was the aim of this study (5).

MATERIALS AND METHODS

The study included 50 patients with ischemic heart disease and a low ejection fraction. The research was conducted at the Clinical Centre in Kragujevac and the Institute for Cardiovascular Diseases "Dedinje" in Belgrade. Patients were subjected to a dobutamine echocardiography stress test (DSE), with the addition of atropine (DASE). All patients were also subjected to coronarography and a revascularisation procedure. After revascularisation lasting for 8 weeks (a period of 2 to 5 months), an assessment of myocardial recovery was conducted.

Analysis of study population.

Inclusion criteria for patients were as follows: optimal ultrasound images, an end-diastolic diameter (EDD) ≥ 6.0 cm (M-mode), an ejection fraction (EF) $<40\%$ (Simpson method) and changes in the coronary blood vessels indicative of significant coronary stenosis ($> 50\%$ diameter stenosis) verified by coronarography. Patients were divided into two groups. The first group consisted of patients with diabetes mellitus, and the second consisted of patients without diabetes mellitus. The group with diabetes mellitus type 2 had a defined glycemic profile and a defined profile of glycolised haemoglobin (HbA1c), as reliable parameters of adequate disease control. Patients were excluded due to the existence of any of the following: congestive heart failure, unstable angina pectoris, significant ventricular arrhythmias, severe valve disease and any contraindications for dobutamine infusion or the administration of atropine (6).

Dobutamine infusion protocol.

The most widely used protocol for DASE, which is used at our institution and is used in this paper, includes the following: after the basic echocardiography study, dobutamine is given intravenously at an the initial dose of $5\mu\text{g}/\text{kg}/\text{min}$ over 3 minutes, which then increases to $10\mu\text{g}/\text{kg}/\text{min}$ in the subsequent 3 minutes, and then again to $20\mu\text{g}/\text{kg}/\text{min}$ and $30\mu\text{g}/\text{kg}/\text{min}$, up to a maximum of $40\mu\text{g}/\text{kg}/\text{min}$, in three-minute intervals. If sub-maximum frequency is not achieved (calculated by the formula $220 - \text{age} \times 0.85$ for men or $200 - \text{age} \times 0.85$ for women), atropine is given in a dose of 0.25 mg , up to a maximum dose of 2 mg . The echocardiogram is continuously monitored. Four views (parasternal long-axis, parasternal short-axis at the papillary muscle level, apical four-chamber, and two-chamber) are recorded at rest, at each stage of dobutamine infusion, and 3 minutes after the termination of infusion. Each of the stages of the test is digitalised directly (on-line) from four sections with the digital system of NovaMicrosonics.

Interpretation of echocardiography findings.

Segmental myocardial contractility was assessed according to the recommendations of the American Society for echocardiography. The left chamber is divided into 16 segments on the basis of these guidelines. The left ventricular wall motion score index (WMSI) is calculated for each stage of the test by summing the points of each segment. The contractility of each segment is determined semi-quantitatively with a score from 1 to 4, with normal contractility segments assessed as 1 ($> 5\text{mm}$ motion of endocardium), hypokinesis assessed as 2 ($< 5\text{mm}$ motion of endocardium), akinesis as 3 (absence of endocardium motion or $< 2\text{mm}$) and dyskinesis as 4 (paradoxical motion to the outside during systole). The characterisation of tissue was made on the basis of segmental wall motion before the test and at low and high dobutamine dosages.



Stress echocardiography.

Before the test and after each stage of the test, echocardiographic records were made on a three-channel electrocardiogram. The ECG was then assessed as normal, ischemic or non-diagnostic. Ischemic changes were defined as the appearance of ST segment depression > 0.1 mV or > 0.2 mV in the leads with ST abnormalities at rest. The electrocardiogram was assessed as non-diagnostic if there was a block of the left branch after digitalis therapy or due to the presence of ST depression > 2 mm at rest.

Coronary angiography.

Visual assessment of stenosis diameter, shown as a percentage, was conducted by two experienced angiographers. Significant coronary stenosis is defined as a reduction of more than 50% in the absolute diameter of the lumen of the main epicardial arteries or their main branches. Patients with verified significant stenosis of at least one major coronary artery were considered to have ischemic dilated cardiomyopathy.

Statistics.

For the purposes of statistical data processing, various methods of descriptive and differential statistics were used. Tables and graphic displays of descriptive statistical methods were produced, as were measures of central tendency and measures of variability in absolute and relative numbers. For the differential statistical analyses, the following methods were used: t-test, χ^2 -test, Mann-Whitney U-test, two-factor analysis of variance with repeated measures and the determination of sensitivity and specificity test. In cases where we had a numerical characteristic of observation that acted according to a normal distribution and two groups of subjects, the t-test was used. In a similar situation with a non-normal distribution, the Mann-Whitney U-test was used. To compare the representation of certain categories of observational characteristics between two or more groups, the χ^2 -test was used. The analysis of the results of the dobutamine stress test obtained in the given times and measurements observed between the two groups of subjects was conducted by a two-factor analysis of variance with repeated measures; in this way, WMSI were tested, as was the average number of segments in the different categories with wall mobility. For comparison of the average number of segments of certain observed categories of wall motion among the two groups, in each of the studied periods of monitoring, a two-factor analysis of variance was performed. On the basis of test results and outcomes after revascularisation, the specificity and sensitivity test was determined.

RESULTS

Clinical and basic echocardiography and electrocardiography information.

The information obtained from the investigated groups is shown in Table 1. Statistically significant differences observed in the groups are not shown when it comes to anthropometrical values and echocardiography data except for body weight and body mass indexes (BMI), which were statistically significantly higher in the group with diabetes mellitus type 2.

NYHA: New York heart Association; NS: not significant.

Baseline data		Ischemic heart disease	Diabetes mellitus and ischemic heart disease	p Value
Mean age (X±SD) (year)		62.7±3.57	60.25±5.52	NS
body weight (X±SD) (kg)		82.27±11.23	89.6±9.99	p=0.022
body high (X±SD) (cm)		174.67±8.86	173.35±6.17	NS
body mass index (BMI)		26.55±2.88	29.88±2.85	p=0.000
gender (n (%))	male	24 (80%)	15 (75%)	NS
	female	6 (20%)	5 (25%)	
NYHA (n (%))	class II	25 (83.3%)	17 (85%)	NS
	class III	5 (16.7%)	3 (15%)	
Ejection fraction		29.6±4.71	29.55±4.3	NS
End diastolic diameter (mm)		66.73±3.82	65.15±3.48	NS
angina (n (%))	yes	19 (63.3%)	9 (45%)	NS
	no	11 (36.7%)	11 (55%)	
family history (n (%))	yes	16 (53.3%)	8 (40%)	NS
	no	14 (46.7%)	12 (60%)	
dyslipidemia (n (%))	yes	17 (56.7%)	16 (80%)	NS
	no	13 (43.3%)	4 (20%)	
smoking (n (%))	yes	17 (56.7%)	11 (55%)	NS
	no	13 (43.3%)	9 (45%)	
hypertension (n (%))	yes	19 (63.3%)	15 (75%)	NS
	no	11 (36.7%)	5 (25%)	

Table 1. Clinical and echocardiographic findings

Stress test results and side effects.

Between the subjects with ischemic dilatative cardiomyopathy with or without glycoregulation disorders, statistically significant differences in the average values of dobutamine dose, atropine, attained sub-maximum frequency, systolic and diastolic blood pressure were not recorded. Statistically significant difference was also not recorded in manifestations such as chest pain, nausea, arrhythmia and changes in the ST segment.

Echocardiographic tracking of LV wall motion results.

Two factorial analyses of variance with repeated measures were used to test the result of of LV-segment classifications at rest, with small doses of dobutamine, with maximal doses of dobutamine and after the test. Individual analyses was made for groups with ischemic dilatative cardiomyopathy with or without glycoregulation disorders. In both of the examined groups, LV function was significantly reduced. The expansion of regional dyssynergy was signifi-



		Baseline	low dose dobutamine	pick dose dobutamine	after revascularisation
Ischemic heart disease	Normal segments(n)	6.67±2.09	10.63±2.27	8.5±2.54	9.83±2.23
	Hypokinetic segments (n)	4.77±2.11	1.87±2.18	3.33±2.72	2.97±2.28
	Akinetic and dyskinetic segments (n)	4.57±1.68	3.5±2.26	4.17±1.91	3.2±2.16
Diabetes mellitus and ischemic heart disease	Normal segments (n)	5.75±2.2	8.65±2.39	6.65±3.3	8.15±2.32
	Hypokinetic segments (n)	4.3±2.9	1.9±2.07	3.1±2.69	2.5±2.12
	Akinetic and dyskinetic segments (n)	5.95±2.63	5.45±2.65	6.25±2.31	5.35±2.62

Table 2. Grading of LV segments per patients at stress test and after revascularisation

cant in both examined groups. At a low dose, dobutamine improves the contractility examined in both groups, with an increasing number of segments marked as normokinetic to account for segments with persistence in kinetic. High doses of dobutamine caused an increase in the number of segments with persistent in kinetic in both examined groups. The test was performed in both groups after myocardial revascularisation using the same protocol (Table. 2). Analysis of certain categories of motion segment classification observed both before the test in relation to the number recorded while using low doses of dobutamine and after revascularisation, as well as in groups of patients with ischemic dilated cardiomyopathy with or without diabetes mellitus, revealed that the number of segments with normal motion was significantly lower before the test. The number of hypokinetic segments was statistically different both before the test in relation to the number recorded while using low doses of dobutamine and after revascularisation, whereas differences were not found when using

high-dose dobutamine in either group. No significant statistical difference was found between the number of akinetic and dyskinetic segments in either of the analysed measurement intervals in both groups (Figure 1). In the group of subjects with coronary disease and diabetes mellitus type 2, the change in type of the number of segments during dobutamine stress testing and after revascularisation was statistically significant (Figure 2). The number of normal segments with small dobutamine doses was the highest; declines were recorded with increasing doses, whereas values increased again after revascularisation in the group with or without diabetes mellitus type II. In all measurement intervals, the number was the highest. The number of hypokinetic segments after revascularisation was lower than with high doses of dobutamine, as well as before the test in the both groups. Akinetic and dyskinetic segments showed the same dynamics, as did hypokinetic segments. The value of WMSI of the observed group was tracked before, during and after the test and after revascularisation. For comparison of the values, two-factor analyses of variance with repeated measures were used. Differences in WMSI values are due to different times of measurement or test phases as well as the period after the revascularisation and are also due to individual differences between the subjects, as well as the differences between the groups. Measurement time in this model is given as an inner group factor, whereas differences between coronary disease with or without the presence of diabetes mellitus are given as a factor of differences between the groups. The response to the test carried out on defined values of WMSI in the observed measurement periods was not significantly different between the tested groups. The level of differences in the observed index values did not change significantly over time, and the difference in WMSI values that existed before the test, during the test and after myocardial revascularisation were approximately the same, as shown in Table 3.

Figure 1. Classification of the segments during the test and after revascularisation in patients without diabetes mellitus type II

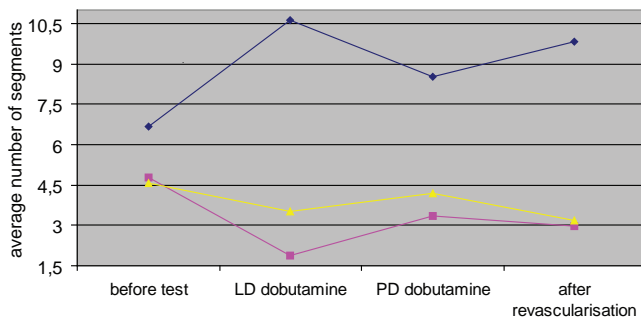
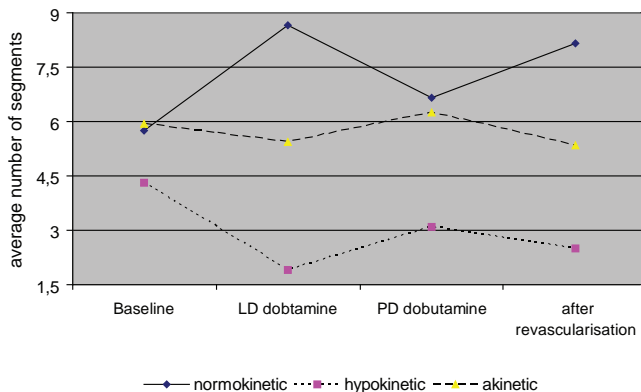


Figure 2. Classification of the segments during the test and after revascularisation in patients with diabetes mellitus type II



	Baseline	LD dobutamine	PD dobutamine	after revascularisation
Ischemic heart disease	1.85±0.27	1.56±0.26	1.74±0.22	1.58±0.24
Ischemic heart disease and diabetes mellitus	1.99±0.32	1.83±0.32	2±0.35	1.86±0.31

Table 3. WMSI per patient at the stress test and after revascularisation



DISCUSSION

Heart attack is the cause of death in about 25% of people in the U.S. and Europe. The mortality rate in patients with deterioration of LV function is growing by 15-60% per year (7). Previous studies (8) have shown a reduction of these rates after revascularisation, with a concomitant improvement in symptoms. After revascularisation, potential benefits must be weighed against the high operating mortality rate in patients with LV dysfunction, which ranges from 5 to 37%. A useful effect of revascularisation is to improve the blood supply of dysfunctional but viable myocardium, with a gradual improvement of regional and total LV function. This is confirmed by a number of studies (9) in which improvements occurred after revascularisation in patients with LV dysfunction but viable myocardial tissue. Previous studies have dealt very little with the role of DSE in the assessment of viable myocardium in the group of patients with low ejection fractions. When it comes to patients with associated long-term diabetes mellitus, this question has been completely unexplored.

In our studied population, a high level of homogenisation has been reached, which creates ideal conditions for the evaluation of diagnostic methods in which only separate variables affect the outcome of the test. The smaller frequency of angina pectoris can be primarily attributed to the existence of autonomic neuropathies. The link between silent coronary events through autonomic neuropathies and lesions of afferent transmission of sensory impulses has been known for long time, as O'Sullivan (1991. Yr.), Jermendy (1993.) and Jalal et al. (1999.) have shown (10). On the other hand, according to findings obtained by selective coronarography, assumptions about the extent of heart disease as a precondition for the development of pain in the analysis of the examined group of patients with diabetes mellitus are invalid. Specifically, Koistinen et al. (1992) (11) have shown by inducing ischemia through efforts in angiography in proven coronary patients that symptomatic patients do suffer from more difficult forms of arteriosclerosis.

Using electrocardiogram analysis, the classification of patients based on previously examined groups according to the presence and location of the Q wave is viewed as a relatively reliable parameter (12) of past coronary events. Conventional techniques used for the determination of viable myocardium include the absence of the Q wave on the EKG, the absence of LV regional dysfunction and fixed scintigraphic perfusion defects. Q view on the EKG is not specific for myocardial infarction; it can be caused by any form of ischemia, under which circumstances it may be reversible. Today, the presence of myocardial thickening as a response to dobutamine infusion observed in akinetic segments, despite the existence of Q view as a sign of IM on EKG, is understood based on pathological studies (91). These studies report that regional contractile dysfunction in relation to the presence of Q view is not always an indicator of transmural scar formation. Bruken (1986.

god.) and Berlinerblau et al. (1994) (13) reported a high prevalence (68%, 72%) of metabolically viable myocardium in regions with Q view. Localisation of Q view in the examined population has shown commonalities among the investigated groups. More frequent appearances on the lower wall among patients in the diabetic group may affect the accuracy of diagnostic tests based on the difficult visualisation of individual segments (14). Rather, homogeneous distributions of coronary heart disease according to the main coronary blood vessels in the investigated groups, with a larger percentage of changes in the RCX and RCA compared to the studies of Pierarda (1990), La Cann (1994) and Piscionea (2001), is a great relief in assessing the predictive value of WMSI and its dynamics during the DSE test, whereas on the other hand it can affect the accuracy of diagnostic methods because of the difficulty in visualising individual segments (15, 16).

From our analysis of blood pressure and cardiac frequency, which are considered the best indicators of cardiac work (17-19), as well as the average value of dobutamine and atropine (20, 21), external events and changes in the final oscillations of EKG, we have confirmed previously established conclusions regarding DASE. The value of dobutamine was not significantly different in the investigated groups, and it ranges through the wide scale of values reported in previous studies (22). Average values for systolic pressure increases fell within the expected range, whereas in hypertonic patients, an increase in systolic pressure was more evident, an effect that has previously been recorded and impacts the sensitivity and specificity of the test (23). Rare changes in the ST segment, registered in several major studies, are a confirmation of the very low sensitivity of ST segment changes with DSE(121). In our work, among the patients with proven multi-vessel coronary disease, reported changes are the expected results. In the group of diabetes patients, the lower representation can be explained by a reduced sensitivity of EKG in these patients and a number of abnormalities while resting that makes the reading more difficult (24).

In the interpretation of regional kinetics while resting, it is likely that mental stress can provoke silent ischemia (25). One-third of all patients who have acute IM with ST elevation never manifested a diagnostic Q view, and 10-15% of IM patients with clinically significant Q view in the period of 2 years leads to his loss. One-third of the total number of IM events remain clinically silent, with approximately 10% belonging to the silent NSTEMI and 5% to silent STEMI that form silent that are lost during time. In the population of patients with diabetes, this percentage is slightly higher than in the general population and may range up to 45% of suffered IM events (26). Mild hypocontractility is often a source of misinterpretation, and in fact is a normal form of a wall motion. Unlike stunned myocardium, which dominates in the early post-infarction period, with chronic myocardial ischemia, hibernated myocardium is dominant as a consequence of the decrease in coronary flow and post-ischemic dysfunction accompanied by



ultrastructural damage of myocytes with a corresponding loss of myofilaments and contractile materials (27, 28). It is clear that this form of dyssynergy at rest dominates in our patient population. Looking at the average number of segments according to the degree of their kinetics, it can be concluded that scar changes are more expanded in the group of patients with diabetes due to a greater average value of the segments with severe kinetic dysfunction. The answer to this situation may be found in the frequency and time of thrombolytic therapy and the degree of coronary heart disease development, i.e., accelerating atherosclerosis and the status of microcirculation (29). This process is accelerated by a smaller number of segments with preserved kinetics in this group. According to the average values of segment kinetics, statistically significant differences have been registered in both groups in the number of normal kinetic segments in relation to the other. The value of WMSI in the group with regular glycoregulation, as well as in the group of patients with diabetes, corresponds to the values published in previous studies (151-153) by authors who treated patients with low EF.

Analysing regional responses between the examined groups' unique tendencies towards improvements in contractility at low doses of dobutamine has been detected, as the expected response taking into consideration that its maximum positive isotropic effect is achieved in this manner. More studies have shown that it is a dose of 7.5 µg/kg/min. By analysing the responses of individual segments that showed improvements at the low dose and were classified in the potentially viable segments, a double tendency was recorded. One group consists of segments with consistent kinetic improvements, whereas the second group consists of segments with bi-phase responses to high doses of dobutamine. New segments with deteriorations in their kinetics have also been registered. Other segments did not show a change in the dynamics of movement during dobutamine infusion. These results correspond to the study of Vigne et al. (30), whereas they minimally deviate from the results of Afridy et al. (31), who were using a continuous increase of 5µg/kg/min in their study. The use of WMSI in the difficult scoring on a four-level scale has influence when there is a diffuse hypocontractility of the chamber.

Three months after complete myocardial revascularisation, a control echocardiogram determined the segments with preserved contractile reserve. Statistically significant differences were noticed in the number of normal kinetics segments in both groups compared to the ones with disturbed kinetics. A tendency towards the movement of segments in the control echocardiography was similar for both groups, and WMSI movement follows this tendency. Identical results were published by Hennessy et al. (32). Unlike the initial papers by Topola et al. (1984.god.), Lazar et al. (1989.god.) and La Cann et al. (1994.god.), in which estimations of viable myocardium were made immediately after myocardial revascularisation, when publishing results with specificity equal to the ones based on later estimations, today it is certain that the right assessment of viable

myocardium should be performed 3 months after the completion of revascularisation procedures (33). The reasons for delayed answers lie in the different criteria for patient selection, the variety of information regarding graft circulation capabilities, different postoperative time periods of evaluation and different methods for wall motion analysis. Moreover, myocardial preservation techniques during surgical revascularisation and the duration of aortic clamping can have an impact on postoperative segmental contractility. Revascularisation techniques need to be taken into account, as do their percentage representation in the tested population. Several factors modify postoperative contractility independently of the procedure. Reductions in postoperative vascular resistance may positively affect kinetics in the early period. It is well known that segments that show improvement with low doses of dobutamine have statistically smaller amounts of fibrotic tissue (34). Additionally, segments in which kinetics are improved after revascularisation have a significantly lower percentage of fibrosis. The highest diagnostic accuracy of DSE is reached by analyses of combinations of continuous improvement and bi-phase response during the test. Alfridy et al, first announced the importance of combining improvements in kinetics with bi-phase response. Kaul (35) suggested that this be the goal of every ischemia provocation test in dysfunctional myocardium because it may be the best predictor of functional repair after revascularisation. Today it is known that it is the response of the myocardium to low doses of dobutamine that is decisive, in contrast to the hypothesis of Armstrong that suggested improvements at any dosage. Constant kinetic improvement during the test, which usually occurs in hypokinetic segments, bears very little predictive value, and the mechanism itself remains unknown (36, 37). Improvements may occur in the field of nontransmural myocardial infarction with the engagement of external tissue in the absence of critical stenosis. Revascularisation of this part of the myocardium does not show any improvement. Functional changes in the border zone of ischemia can also affect the test results. Recent research indicates that the area of dysfunction is spread over more than 30% or 1 cm outside the ischemic zone. In this way, dobutamine enhances the contractility of non-ischemic tissue that is characterised as necrotic. To overcome this problem, the principle to ignore improvements in the area up to 1cm around the affected tissue is used.

DASE showed that the sensitivity in diagnosing viable myocardium in the group of ischemic dilated cardiomyopathy patients was 95% in our study. The results of several studies, ranging from 68-100% (38). In the group of ischemic dilated cardiomyopathy and diabetes mellitus type 2 patients, the sensitivity of the test was 93%. The sensitivity given by the work of France et al. was 86%. Test sensitivity analysis is necessary to consider all aspects of the implementation of the echocardiography stress test, i.e., the method of titrating dobutamine during the test, especially at low doses of 2.5, 5 or 10µg/kg/min-duration. Other factors include the adequacy of the ultrasound de-



vice, variations in the cine loop technique and, perhaps the most important factor in the implementation of a test: the human factor, i.e., the training and experience of the echocardiographer (39, 40). An important role is given to the level of coronary heart disease, the degree of coronary artery stenosis, i.e., a relatively small percentage of viable tissue, up to 35% below the occluded coronary artery with reduction of sensitivity methods (52%) (41), the existence and degree of residual flow after the IM, myocardial wall thickness, the state of microcirculation and the degree of fibrosis (42). An important fact in our work is the high selectivity when creating the group of observed patients. This approach is less selective when creating a group after thrombolysis as elaborated by Smart, who showed that the improvement of WMSI with a small dose of dobutamine had a sensitivity of 71% in relation to recovery during the observation, whereas the lack of progress had a 74% specificity in relation to the improvement in the observation period. These investigators also found that improvements at the low doses of dobutamine up to 20 μ g/kg/min were not a real indicator of recovery. Whereas the current study indicates that improvements in regional function following dobutamine administration have predictive value regarding the viability of myocardial segments in the early post-infarction period, several results support or deny the effectiveness of dobutamine testing with regard to myocardial viability in the long run, after the IM.

In our work, the DASE showed a 91% specificity in the group of patients with ischemic dilated cardiomyopathy, whereas in the group with diabetes mellitus type 2 this value was 96%. This is a relatively high specificity in relation to the results of other studies (43-45). Echocardiography testing with a high dose of dobutamine, the addition of atropine and the provocation of ischemia, i.e., bi-phase response, do reduce the sensitivity of the test; however, as it is well known, these methods also increase the specificity of the test. It is also known that the contractility of border areas around the ischemic zone impacts the decrease in sensitivity; even 40% akinetic and 5% dyskinetic segments that showed kinetic improvements during the test are a consequence of nontransmural IM and have a completely normal histological material. Low EF and multi-vessel coronary heart disease, in the absence of the significant influence of diabetes mellitus in test feasibility, produces actual sensitivity and specificity. The improvement in regional function of the chamber is not the main signal for intervention, which is actually the real objective, while improving the global functions of LV and patients status. These final results can be measured by ventricular volume and EF or working capacity after revascularisation.

The viability of the myocardium represents a deterioration in contractile function that is potentially reversible if adequate resaturation of the coronary circulation takes place. Deterioration may be the result of various causes, including acute myocardial ischemia, myocardial hibernation, acute myocardial stunning or repeated stunning (46). The difference between these pathophysiological entities

is probably not relevant to the clinical aspects of the disease, as not only does this situation often coincide, but the treatment would represent complete revascularisation. An accurate diagnosis of viable but not contractile myocardium in patients with ischemic heart disease and preceding IM allows the application of treatment on the basis of heart failure symptoms and angina pectoris while also taking into account the effects of revascularisation. Recent views suggest that, whereas the level of LV dysfunction is the main parameter for prognosis, LV dysfunction is not necessarily irreversible and can be improved after the procedure of myocardial revascularisation. Patients with significant LV dysfunction can benefit from bypass surgery or percutaneous coronary interventions the most because they are at the highest risk in the event of further loss of contractile tissue mass; however, they also have the highest level of mortality with respect to surgical procedures. When we discuss surgical revascularisation of the myocardium, its adequacy should be taken into account (graft occlusion and restenosis), as should the existence of associated myocardial diseases, LV hypertrophy, and myopathic processes. Recent studies have taken into account the global function of LV, physical capacity, quality of life and death rate as their most important parameters. Other factors that are taken into account in the definition of diagnostic tests are feasibility, accessibility and experience of the test performer (47-53).

LIMITATIONS OF THE RESEARCH

Our study had several limitations. Firstly, the number of patients tested was small. Secondly, coronarography results were not compared with starting echocardiography studies in terms of the comparison of myocardial dysfunction with the existence of residual flow and occluded coronary arteries. Thirdly, the effect of silent ischemia on the segmental contractility, especially in the diabetic group. Moreover, there are a number of restrictions when it comes to the interpretation of echocardiography studies. Visualisation and segmental analysis of large chambers, as is the case with our patients, is always difficult. The cine loop allows researchers to monitor and compare segments of the wall next to one another, but the selection of identical segments is the responsibility of the echocardiographer. The timing of the cardiac cycle is very important and any error in the recording start makes the loop useless. Attention should be given to the interpretation of data from the basal segment of the inferior wall, due to the overlap with echoes from the valve or even atrium chamber wall. Additionally, the images taken in parasternal cross-section should be carefully interpreted due to the presence of artefacts. It is necessary to visualise the apex effectively, as it is the most common place for abnormalities (54-56). By using high-dose dobutamine and provoking ischemia, we have reduced the sensitivity of the test. We did not use a dose of 7.5 μ g/kg/min as our starting dose. Factors such as post-operative disturbed sympathetic tone, heart frequency,



aortic pressure and overfilling of the LV can influence heart movements. Very frequent appearance of abnormal movement of the septum after surgical intervention is also a problem. Finally, the echocardiography study was conducted over a wide time range after revascularisation (2-5 months). It is widely accepted that the golden standard is 3 months (57-59).

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INTRAVENOUS IMMUNOGLOBULIN ATTENUATES DIABETES INDUCTION IN MICE

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INTRAVENSKI IMUNOGLOBULINI INHIBIRAJU RAZVOJ DIJABETESA KOD MISEVA

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ABSTRACT

Type 1 diabetes mellitus is an autoimmune disease in which pathologic autoreactive T cells attack the insulin-secreting pancreatic islets of Langerhans. Intravenous immunoglobulin has been shown to have a therapeutic effect in some autoimmune diseases. The therapeutic effect of intravenous immunoglobulin has not been tested in type 1 diabetes mellitus models.

Objectives: We examined the effect of intravenous immunoglobulin (IVIG) on the development of immune-mediated diabetes induced by administration of multiple low doses of streptozotocin (SZT) in susceptible C57BL/6 male mice.

Methods: Diabetes was induced by five daily injections of streptozotocin. Mice were treated daily with either 50 mg/kg or 200 mg/kg body weight IVIG for 15 days. Control animals received equivalent doses of human serum albumin. Glycaemia and glycosuria were evaluated daily, and serum levels of TNF α , IL-17 and HbA1c were determined on day 21.

Results: Assessment of glycaemia ($p < 0.001$), glycosuria ($p < 0.01$), and HbA1c levels demonstrated that treatment with 200 mg/kg IVIG significantly attenuated diabetes induction. Calculation of Pearson's correlation coefficients indicated an inverse correlation between HbA1c levels and IVIG dose ($p < 0.04$). Finally, serum levels of TNF α and IL-17 were significantly lower in IVIG-treated mice than in control mice ($p < 0.05$).

Conclusion: Our results show for the first time that IVIG may attenuate diabetes induction by reducing serum levels of proinflammatory cytokines.

Keywords: Diabetes mellitus, intravenous immunoglobulin, TNF α , IL-17

SAŽETAK

Dijabetes melitus tip 1 je autoimuna bolest u kojoj patološki, autorektivni T limfociti oštećuju Langerhansova pankreasna ostrvca. Pokazano je da intraveniski imunoglobulini imaju terapijski efekat kod mnogih autoimunih bolesti. Terapijski efekat imunoglobulina u modelu dijabetes melitusa tip 1 nije ispitivan. Ciljevi: Mi smo ispitivali efekat intravenskih imunoglobulina na razvoj dijabetes melitusa izazvanog niskim ponovljenim dozama streptozotocina kod osetljivih C57BL6 miševa.

Metode: Dijabetes je izazivan sa pet dnevnih injekcija streptozotocina. Korišćene su dve doze intravenskih imunoglobulina (50mg/kg, i 200mg/kg telesne mase) svakodnevno 15 dana. Kontrolne životinje dobijale su istu dozu humanog serumskog albumina. Glikemija i glikozurija mere su dnevno, dok se nivo serumskog TNF α i IL-17, kao i nivo HbA1c određivao 28.dana

Rezultati: Primena većih doza intravenskih imunoglobulina (200 mg/kg,) značajno je smanjila pojavu dijabetesa, što je praćeno kroz vrednosti glikemije ($r < 0,001$), glikozurije ($r < 0,01$) i nivoa HbA1c. Pirsonova korelacija pokazala je inverznu korelaciju između nivoa HbA1c u krvi i doze intravenskih imunoglobulina ($R < 0,04$). Konačno, serumski nivo TNF α i IL-17 je bio značajno snižen nakon primene intravenskih imunoglobulina ($r < 0,05$).

Zaključak: Naši rezultati su po prvi put pokazali da primena intravenskih imunoglobulina suprimiraju indukciju dijabetesa verovatno smanjenjem nivoa serumskoih proinflamatornih citokina.

Ključne reči: Dijabetes melitus, intraveniski imunoglobulini, TNF α , IL-17



INTRODUCTION

Intravenous immunoglobulin (IVIG) has been used for the treatment of patients with primary and secondary antibody deficiencies [1] and more recently has been proven to be beneficial in autoimmune and systemic inflammatory diseases [2-4]. IVIG may be an effective treatment for a range of diverse immunological conditions due to its wide-ranging modes of action [5].

IVIG may be considered to have four separate mechanisms of action: a) actions mediated by the variable regions F(ab')₂, b) actions of Fc on a range of Fc receptors (FcR), c) actions mediated by complement binding within the Fc fragment, and d) immunomodulatory substances present in the preparation of IVIG [6]. Through these mechanisms, IVIG may inhibit the function of autoreactive T cells, suppress harmful autoantibodies through anti-idiotypic interactions, and interfere with the production of proinflammatory cytokines [7-9]. Due to these anti-inflammatory and immune modulating features, IVIG has proven useful in the management of autoimmune disorders [10-14].

We have evaluated the effect of human IgG for i.v. use on the onset and progression of diabetes mellitus, a T cell-mediated autoimmune disease.

Diabetes mellitus type 1 is a chronic inflammatory disorder characterised by the autoimmune destruction of pancreatic β cells. This breakdown of immunological self-tolerance results in autoreactivity to islet self-antigens [15]. Immunity and inflammation play an important role in β cell destruction through the recruitment and activation of T cells and macrophages to pancreatic islets and the local production of inflammatory cytokines [16,17]. The cytokine network is a multi-faceted system that is regulated by a delicate balance between two distinctive cytokine pathways [18]. In general, the balance between Th1-type and Th2-type cytokines, which are locally produced in pancreatic islets by immune cells, is crucial to diabetes development [19,20]. Thus, a bias towards the Th1-type cytokines IFN γ and TNF α promotes inflammatory insulinitis and diabetes [21,22], whereas a bias towards the Th2-type cytokines IL-4 and IL-10 prevents beta-cell destruction in NOD mice [23,24,25]. Type 1 cytokines initiate a cascade of inflammatory processes in the islets (insulinitis), culminating in beta-cell destruction [26]; however, proinflammatory cytokines such as interleukin 1 (IL-1) and tumour necrosis factor (TNF α), both produced by macrophages, are involved in pancreatic islet β cell functional suppression and death [27]. Recent studies have suggested that Th17 may play an important role in cell-mediated autoimmune inflammatory diseases [28] such as EAE [29] and diabetes mellitus [30,31]. IL-17-producing effector T cells (Th17 cells) are efficient inducers of tissue inflammation and crucial initiators of organ-specific autoimmunity. The activity of Th17 cells is associated with the induction of proinflammatory cytokines such as TNF α , IL-1, IL-6, and IL-8 and with enhanced proliferation, maturation,

and chemotaxis of neutrophils [32]. The induction of Th17 cells in the secondary lymphoid tissue and the maintenance of these cells in the target organ are essential in initiating autoimmune tissue inflammation [33]. The type and intensity of inflammation depend on the balance of cytokines produced; Th1 and Th17 cytokines promote inflammation, and Th2 and Treg cytokines inhibit inflammation. During this period, an imbalance in β cell damage [34] and β cell repair [35] results in decreases in the number and function of β cells, eventually culminating in overt hyperglycaemia.

We analysed the effects of IVIG in the MLD-STZ-induced mouse model of diabetes mellitus [36], in which low-dose STZ treatment results in a cellular immune attack against β -cells that have been rendered antigenic. The aim of this study was to evaluate the antidiabetogenic potential of IVIG in this model and to define the possible mechanisms of action of IVIG.

In this study, we have shown that treatment of C57BL/6 mice with intravenous IgG attenuates MLD-STZ-induced diabetes mellitus and reduces serum levels of proinflammatory cytokines.

MATERIALS AND METHODS

Experimental animals. The experiments were approved by the ethics board of the Medical Faculty of Kragujevac. Eight- to ten-week-old male C57BL/6 mice weighing between 18 and 24 g were used for all experiments. The mice were housed in our laboratory animal facility under standard conditions. Mice were divided into three groups. The first group was treated with 50 mg/kg IVIG, the second group was treated with 200 mg/kg IVIG, and the third group was treated with human serum albumin.

Diabetes induction. Streptozotocin (STZ, Sigma Chemical, St. Louis, MO) was dissolved in citrate buffer, pH 4.5 and administered intraperitoneally at a dose of 40 mg/kg/day for five consecutive days.

IVIG The preparation of IVIG (Bulbio, Sofia) was provided by The Stephan Angelov Institute of Microbiology (Sofia, Bulgaria) (immunoglobulin concentration, 50 mg/ml). Beginning on the day of the STZ immunisation, mice received daily subcutaneous injections of 50 mg/kg or 200 mg/kg IVIG for 15 days.

Human serum albumin administration Control animals received human serum albumin. Human serum albumin was dissolved in sterile 0.9% NaCl and administered subcutaneously at a dose of 200 mg/kg/day for 15 days.

Diabetes evaluation: Daily blood glucose (Abbott Exccide) determination was performed on samples taken from the tail tip after starvation for four hours. Daily urine glucose levels were analysed every day using Uroscan 2 test strips. Levels of glycosylated haemoglobin (HbA1c; Olympus, Center Valley, Pennsylvania) in blood were determined on day 28. Paraffin-embedded sections were stained with haematoxylin and eosin (H&E) following standard protocols for light microscopic examination.



Histological examination

Pancreata of mice in each group were excised and placed in 10% buffered formaldehyde fixative solution overnight at room temperature. For quantitative histology of infiltrating cells, 10% buffered formaldehyde-fixed pancreata were embedded in paraffin wax, and paraffin-embedded sections were stained with H&E following standard protocols for light microscopic examination. Original H&E-stained slides were reviewed to confirm the presence of infiltrating lymphocytes in the pancreas.

Measurement of mouse TNF α and IL-17 in sera by sandwich ELISAs

Sera were collected by single needle stick and stored at -20 °C until analysis. IL-17 and TNF α concentrations were measured using highly sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) specific for the mouse cytokines according to the manufacturer's instructions.

Briefly, premixed standards were reconstituted in PBS, pH 7.2, generating stock concentrations of 2000 pg/mL for TNF α and 1000 pg/mL for IL-17. The standard stocks were serially diluted in reagent diluent to generate seven points for the standard curves.

Diluted capture antibody was added to a 96-well flat-bottomed polystyrene microtitre plate (MTP) in a final volume of 100 μ L. Plates were sealed, incubated overnight at room temperature, and washed with wash buffer using an autowasher. The samples were diluted 1:4 in reagent diluent. Premixed standards or diluted samples (100 μ L) were added to each well containing washed beads, covered with an adhesive strip and incubated for two hours at room temperature. After incubation and washing, 100 μ L of the premixed detection antibody was added to each well, wells were covered with a new adhesive strip, and samples were incubated for two hours at room temperature. After incubation and washing, streptavidin-HRP was added to each well (100 μ L), and samples were incubated for 20 min at room temperature in the dark. After washing, the beads were re-suspended in 100 μ L of substrate solution, 50 μ L of stop solution were added to each well, and the optical density of each well was determined immediately using a microplate reader set to 450 nm.

Statistical analysis

The data were analysed using SPSS version 13 statistical package. Normally distributed data were compared using Student's *t*-tests, and non-normally distributed data were compared using Mann-Whitney tests.

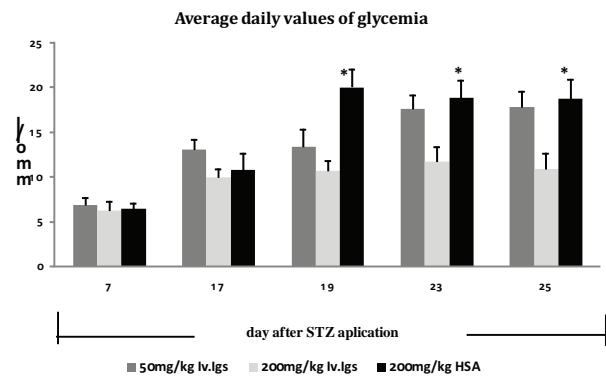
RESULTS

1.1 Glycaemia

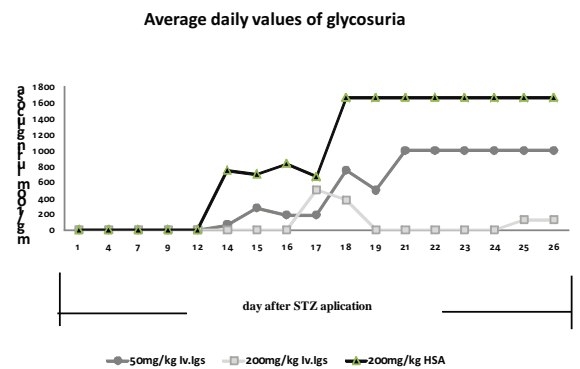
High doses of IVIG decrease glycaemia in susceptible C57BL/6 male mice with low dose streptozotocin (STZ)-induced immune-mediated diabetes

Mice were divided in two groups. One group was treated with 50 mg/kg IVIG, and the other was treated with 200 mg/kg IVIG for 15 days. Control animals received equivalent doses of human serum albumin (HSA). The mice treated with multiple low doses of STZ and HSA gradually

A



B



C

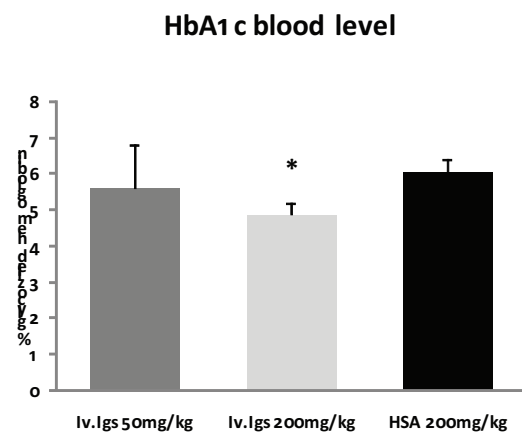


Fig.1. IVIG induces significant attenuation of diabetes induction A. Treatment with a high dose of IVIG had significant antidiabetogenic effects, as demonstrated by attenuation of glycaemia ($p < 0,05$ from day 19 until the end of the experiment). B. Treatment with 200 mg/kg IVIG resulted in significantly decreased glycosuria ($p < 0,05$ on day 18), and urine glucose levels remained low in mice treated with 200 mg/kg IVIG until the end of the experiment. C. HbA1c, which is proportional to the level of glucose in the blood throughout the current erythrocyte life cycle and therefore serves as an indicator of an extended diabetic state, was present at significantly lower levels in mice treated with 200 mg/kg IVIG than in control mice ($p < 0,05$).

became hyperglycaemic. Treatment with 200 mg/kg IVIG resulted in a significant decrease in blood sugar (Fig.1.A; $p < 0.05$ by day 19); however, treatment with 50 mg/kg IVIG did not significantly alter the development of glycaemia associated with MLD-STZ-induced diabetes.

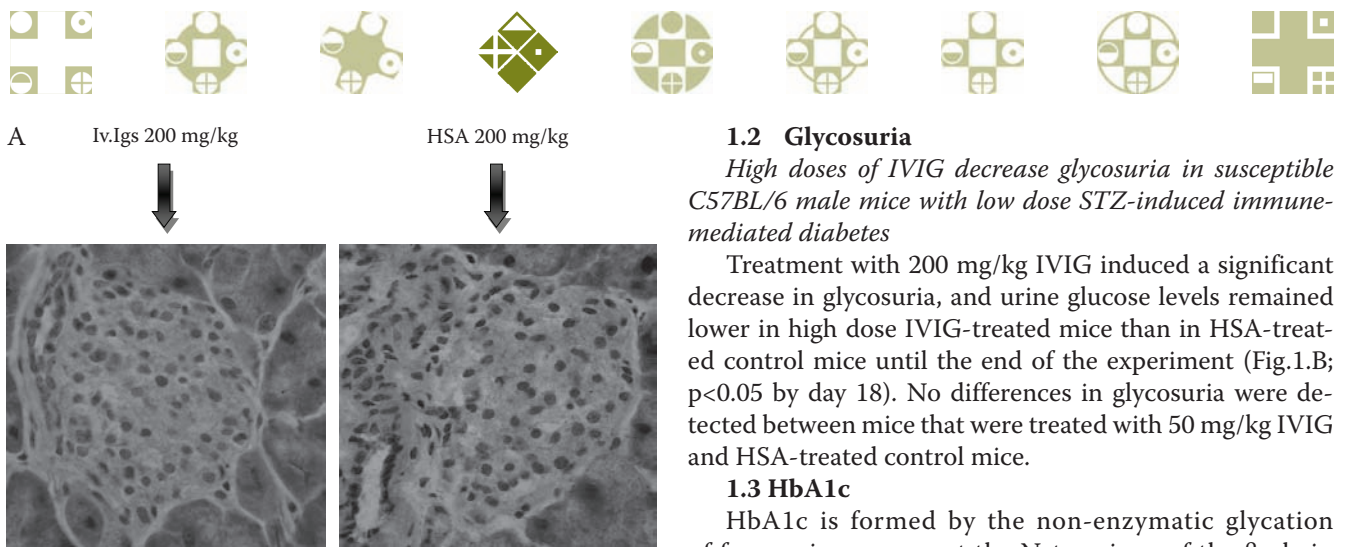


Fig.2. IVIG induces significant attenuation of diabetes induction A. Analysis of the mononuclear cell infiltrates in the pancreatic islets of MLD-STZ-treated mice demonstrate milder mononuclear cell infiltration in mice treated with 200 mg/kg IVIG.

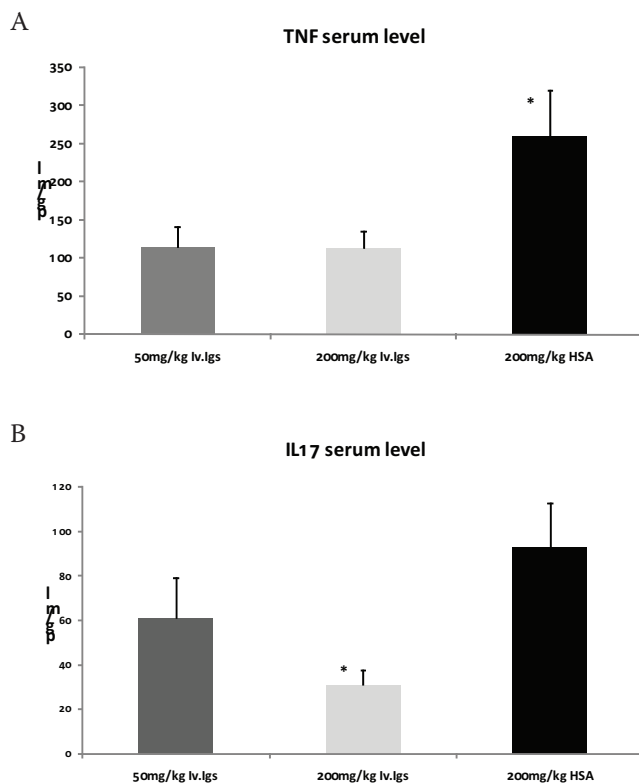


Fig.3. High doses of immunoglobulins significantly decrease serum levels of proinflammatory cytokines in C57BL/6 mice with immune-mediated diabetes mellitus induced with multiple low doses of streptozotocin:

A. Serum levels of TNF- α in IVIG-treated mice (114.7 \pm 27,13 pg/ml for 50 mg/kg IVIG, 112.7 \pm 22,97 pg/ml for 200 mg/kg IVIG) were 2.3-fold lower than those in HSA-treated control mice (260,8 \pm 59,79 pg/ml) by day 28. B. Although both doses of IVIG reduced the level of IL-17, only 200 mg/kg IVIG resulted in a statistically significant decrease in the serum IL-17 level (60.9 \pm 20,3 pg/ml for 50 mg/kg IVIG, 30.6 \pm 6,8 pg/ml for 200 mg/kg IVIG) compared with the levels in mice treated with HSA (92.8 \pm 24,8 pg/ml).

1.2 Glycosuria

High doses of IVIG decrease glycosuria in susceptible C57BL/6 male mice with low dose STZ-induced immune-mediated diabetes

Treatment with 200 mg/kg IVIG induced a significant decrease in glycosuria, and urine glucose levels remained lower in high dose IVIG-treated mice than in HSA-treated control mice until the end of the experiment (Fig.1.B; $p < 0.05$ by day 18). No differences in glycosuria were detected between mice that were treated with 50 mg/kg IVIG and HSA-treated control mice.

1.3 HbA1c

HbA1c is formed by the non-enzymatic glycation of free amino groups at the N-terminus of the β -chain of haemoglobin A0. The level of HbA1c is proportional to the level of glucose in the blood throughout the current erythrocyte life cycle. Increased levels of HbA1c reflect glycaemia over a longer time interval. Glycosylated haemoglobin (HbA1c) blood levels were determined on day 28. We observed a statistically significant positive correlation between daily average glycaemia ($R^2 = 0,5466$; $p < 0,01$), daily average glycosuria ($R^2 = 0,9704$; $p < 0,001$) and HbA1c level. HbA1c blood levels were significantly lower in mice treated with 200 mg/kg IVIG than those in mice treated with HSA (Fig.1.C; $p < 0.05$); however, treatment with 50 mg/kg IVIG did not ameliorate glycaemia.

2.1 Protection from insulinitis in IVIG-treated mice

Morphological examination of the pancreas of mice receiving STZ and HSA on day 28 revealed obvious insulinitis and structural changes of the islets (Fig.2A). A similar histological pattern was found around the islets in mice treated with 200 mg/kg IVIG, but the number of inflammatory lesions and the degree of insulinitis appeared to be greatly decreased (Fig.2A).

3.1 IVIG affects serum levels of proinflammatory cytokines after MLD-STZ diabetes induction

To study the effects of immunoglobulin treatment on secreted cytokine profiles, we quantified the levels of TNF α and IL-17 in the sera of treated and control mice by ELISA on day 28. Treatment of mice with IVIG caused a reduction in TNF α and IL-17 levels. Serum levels of TNF α in mice treated with either 50 mg/kg or 200 mg/kg IVIG were lower than those in mice treated with HSA (Fig.3.A; $p < 0.05$); however, only treatment with 200 mg/kg IVIG resulted in a significant decrease in serum levels of IL-17 (Fig.3.B; $p < 0.05$).

DISCUSSION

Intravenous immunoglobulin treatment has been reported to be beneficial in a large number of autoimmune diseases that are mediated by either antibodies or by T cells. In the present study, we have shown for the first time that IVIG (200 mg/kg) mitigates the development of disease in an MLD-STZ model of type 1 diabetes by demonstrating attenuation of glycaemia, glycosuria and increased HbA1c



levels. Previous studies have suggested that IVIG prevents T cell infiltration into the central nervous system (CNS), thus preventing the onset of irreversible neurologic damage and inhibiting the induction of EAE [37,38]. IVIG has also been reported to have a beneficial effect during the development of adjuvant arthritis [28]. Similarly, Fangqi et al. [39] observed a therapeutic effect of IVIG treatment in experimental autoimmune myocarditis. Furthermore, IVIG has been shown to be an effective treatment in a number of autoimmune neurological disorders [5].

Modulation of the production of cytokines and cytokine antagonists by IVIG is a major mechanism by which immunoglobulin exerts its anti-inflammatory effects in vivo [40] in various inflammatory and autoimmune diseases. We found that a high dose of IVIG (200 mg/kg) decreased the number of inflammatory lesions and the degree of insulinitis.

Studies over the past decade have strengthened the association between pro-inflammatory cytokines and several organ-specific autoimmune diseases including type 1 diabetes [41]. The balance between Th1 and Th2 cytokines has been shown to be disrupted in many autoimmune disorders in which Th1 cytokines predominate over counter-regulatory Th2 cytokines [42]. Type 1 diabetes is an autoimmune condition associated with the T cell-mediated destruction of pancreatic β cells. Furthermore, the functional imbalance between the two Th cell subsets is a key determinant in establishing islet pathology [43,44]. Recent studies have indicated that Th1 populations are key mediators of β cell autoreactivity, while induction of Th2 populations results in a dominant protective effect. β cell-destructive insulinitis is associated with increased expression of proinflammatory cytokines (IL-1 and TNF α), and pancreatic islet β cell destruction and type 1 diabetes, like other organ-specific autoimmune diseases, result from a disorder of immunoregulation [45]. Marked increases in the expression of IL-1, TNF α , IFN γ , IL-6 and IL-12 mRNA have been demonstrated in the insulinitis infiltrates of NOD and BB islets in vivo [46-50]. Dunger et al. [51] demonstrated that in vitro treatment of islets with TNF α , IFN γ or a combination of TNF α and IFN γ inhibited glucose-stimulated insulin secretion in a dose-dependent manner. MLD-STZ similarly upregulated IFN γ and TNF α at both the protein and gene levels in islets of C57BL/6 mice [19] and C57BL/6KsJ mice [52]. Similar conclusions were drawn from results obtained in patients at onset of type 1 diabetes [53]. Recent studies suggest that Th17 cells are involved in the pathogenesis of autoimmune diabetes [54]. High levels of the IL-17 transcript have been found within insulitic lesions in NOD mice, and increased levels of serum IL-17 were associated with the development of diabetes in a T cell receptor transgenic NOD model with accelerated disease progression [55]. More recently, studies have demonstrated that therapeutic intervention with an antigen-specific agent that protects against diabetes in NOD mice is associated with a decrease in Th17 populations [56].

Several groups have demonstrated that IVIG can help to restore the cytokine balance [57]. IVIG inhibits the differentiation of Th0 cells into Th1 cells and enhances the differentiation of Th0 cells into Th2 cells [58]. We have observed the decreased production of the proinflammatory cytokines TNF α and IL-17 after IVIG treatment in MLD-STZ-induced diabetes mellitus. IVIG treatment has been shown to result in decreased production of the inflammatory cytokine TNF α in two experimentally induced T cell autoimmune diseases in Lewis rats: experimental autoimmune encephalomyelitis and adjuvant arthritis [28,37]. Bayry J et al. [59] also demonstrated the ability of IVIG to inhibit cytokine production by immature dendritic cells; however, production of TNF α by LPS-stimulated dendritic cells was found to be unaffected by IVIG. Decreased TNF α serum production was also observed in the experimental autoimmune myocarditis model when BALB/C mice were treated with IVIG [39]. Furthermore, the ability of IVIG to neutralise proinflammatory cytokines such as TNF α was demonstrated to be one of the important mechanisms by which IVIG is therapeutically beneficial in rheumatoid arthritis (RA) and Kawasaki disease in humans [60].

Our results show for the first time that IVIG may attenuate diabetes induction. This effect is accompanied by decreased production of proinflammatory cytokines, which is also associated with a decreased number of inflammatory lesions in Langerhans islets.

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CARDIO-RENAL SYNDROME - DEFINITION, CLASSIFICATION AND BASIC PRINCIPLES OF THERAPY

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KARDIO-RENALNI SINDROM: DEFINICIJA, KLASIFIKACIJA I OSNOVNI PRINCIPI LEČENJA

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ABSTRACT

Cardio-Renal Syndrome is defined as pathophysiological dysfunction of the heart and kidneys in which an acute or chronic abnormality of one organ favours either the acute or chronic disorder of the other one. Due to complex interactions between the heart and kidneys, Cardio-Renal Syndrome is divided into five types: Cardio-Renal Syndrome type 1 (Acute Cardio-Renal Syndrome) is defined as acute kidney dysfunction as a result of acute heart failure. Cardio-Renal Syndrome type 2 (Chronic Cardio-Renal Syndrome) is a progressive chronic kidney disorder as a result of chronic heart failure. Cardio-Renal Syndrome type 3 (Acute Reno-Cardial Syndrome) is defined as an acute heart abnormality due to acute kidney dysfunction. Cardio-Renal Syndrome type 4 (Chronic Reno-Cardial Syndrome) is defined as a chronic heart function disorder as a result of a chronic kidney disorder. Cardio-Renal Syndrome type 5 (Secondary Cardio-Renal Syndrome) is defined as either a permanent or temporary abnormality of both organs due to systemic disease. Early detection of kidney and heart disease and timely and adequate therapy can prevent the development of Cardio-Renal syndrome and reduce the overall expense of therapy.

Keywords: *cardiorenal syndrome, acute heart failure, acute kidney injury, chronic kidney disease, chronic heart failure*

SAŽETAK

Kardio-renalni sindrom se definiše kao patofiziološki poremećaj srca i bubrega, u kome akutni ili hronični poremećaj funkcije jednog organa podstiče akutni ili hronični poremećaj drugog organa. Zbog složene interakcije srca i bubrega razlikuju se pet tipova kardio-renalnog sindroma. Kardio-renalni sindrom tip 1 (akutni kardio-renalni sindrom) se definiše kao akutno oštećenje bubrega nastalo usled naglog pogoršanja funkcije srca. Kardio-renalni sindrom tip 2 (hronični kardio-renalni sindrom) se definiše kao progresivno hronično oštećenje bubrega koje nastaje usled hroničnog poremećaja funkcije srca. Kardio-renalni sindrom tip 3 (akutni reno-kardijalni sindrom) se definiše kao akutno oštećenje funkcije srca koje nastaje kao posledica akutnog oštećenja bubrega. Kardio-renalni sindrom tip 4 (hronični reno-kardijalni sindrom) se definiše kao hronično oštećenje funkcije srca koje nastaje usled hroničnog oštećenja funkcije bubrega. Kardio-renalni sindrom tip 5 (sekundarni kardio-renalni sindrom) se definiše kao povratno ili nepovratno oštećenje oba organa nastalo usled sistemskih bolesti. Rano otkrivanje bolesti srca i bubrega, i pravovremena primena odgovarajućeg lečenja sprečavaju razvoj kardio-renalnog sindroma i smanjuju troškove lečenja ovih bolesnika.

Ključne reči: *kardio-renalni sindrom, akutna srčana slabost, akutno oštećenje bubrega, hronična bolest bubrega, hronična slabost srca*

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INTRODUCTION

Cardio-Renal Syndrome (CRS) is defined as a pathophysiological disorder of the heart and kidneys in which an acute or chronic disorder of one organ favours the acute or chronic disorder of the other [1-6]. Direct and indirect actions due to the insufficiency of one organ may trigger damage to the other via a complex combination of neurohumoural feedback mechanisms [1-6].

METHODS

The MEDLINE database was used for this study. The following keywords were used for searching: cardio-renal syndromes, acute heart failure, acute kidney injury, worsening renal function, chronic kidney disease, chronic heart failure. About 10000 references were found dealing with these types of problems. Systematic review articles and well controlled clinical studies were extracted. Editor's letters and uncontrolled clinical studies were not used. Systematic review articles were used and analysed by the Acute Dialysis Quality Initiative (ADQI) consensus group, which checked the validity and quality of the selected references.

RESULTS

Types of cardiorenal syndrome

Due to the complex interactions of the heart and kidney, cardiorenal syndrome is divided into five categories [1-6].

Cardiorenal syndrome type 1

Cardiorenal syndrome type 1 (acute cardiorenal syndrome) is defined as acute renal failure caused by sudden worsening of heart function, such as hypertensive pulmonary oedema with preserved systolic function, acute decompensation of chronic congestive heart failure, acute cardiogenic shock and predominant right ventricle failure [1-7].

In acute heart failure (including acutisation of chronic decompensatory heart failure, heart arrhythmia or ischaemia) compromised cardiac output is reflected by an impairment in the strength of glomerular filtration and acute renal failure [acute renal hypoperfusion, lowered oxygen income, apoptosis and necrosis of renal cells, lowered strength of glomerular filtration and inadequate response to natriuretic peptides (ANP - atrial natriuretic peptide, BNP - brain natriuretic peptide)]. A sudden drop in intravascular volume in the renin-angiotensin-aldosterone system (RAAS) and excessive angiotensin 2 stimulates the creation and deliberation of endothelin-1 (ET-1) in the kidney. Endothelin-1 is a potent proinflammatory and profibrotic vasoconstrictive peptide that plays an important role in most pathological mechanisms of acute renal failure by activating an ischaemic cascade during secondary acute renal damage [1-7]. Patients with CRS type 1 have

higher concentrations of lipocaline (lipocaline linked with neutrophil gelatinisation) in their urine, indicating the incipient development of renal impairment [1, 8].

The basic goal of treatment for CRS type 1 is the stabilisation of impaired heart function, which is the main cause of hemodynamic instability, and generation of better renal perfusion [7]. Acute renal impairment with or without hyperkalaemia has an impact on the choice of drugs used for treatment; angiotensin-1 convertase blockers, angiotensin-1 receptor blockers and aldosterone blockers can all raise the survival of patients with heart failure and myocardial infarction. Monitoring of renal function and serum potassium concentration is necessary in patients with CRS type 1. Acute administration of β -blockers in CRS is not advised. Blockage of inotropic compensation, which is dependent on compensatory tachycardia and on the sympathetic nervous system, can worsen cardiogenic shock [1-7].

In the case of hypovolaemia, eliminating excessive body water can lead to changes in the diuretics used and, in certain situations, require the adjustment of dialysis treatment modality (SCUF - slow continuous ultrafiltration) [5-7]. Adding diuretics that block distal tubules (chlorthiazide 500-1000 mg i.v. or metolazone 2.5-10 mg per os) can significantly improve diuresis in patients who receive diuretics of Henle's loop [7, 9].

Nitrate doses should be titrated with the goal of lowering blood pressure by 10 mmHg while at the same time maintaining systolic blood pressure above 100 mmHg. The dosage of nitrates should be lowered if systolic blood pressure is between 90 and 100 mmHg and if blood pressure falls below 90 mmHg, nitrate use should be discontinued [7, 9].

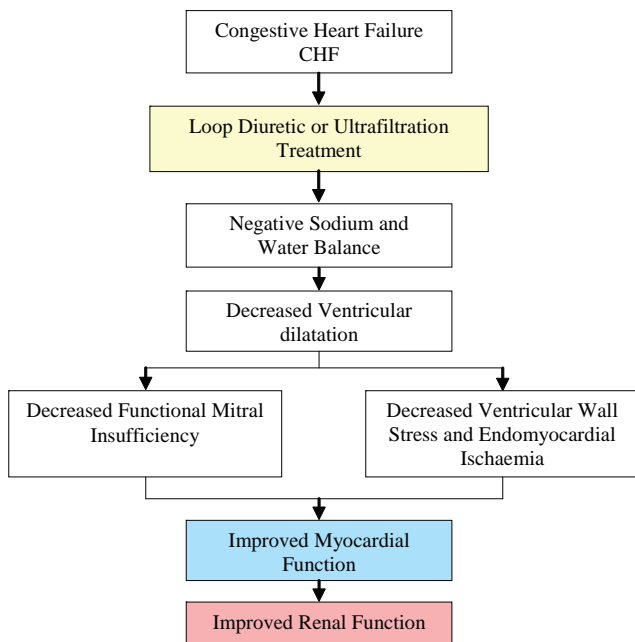
Inotropic therapy is used in patients with acute heart failure and systolic blood pressure below 90 mmHg [7, 9]. For the vast majority of patients, dobutamine at a dose of 1.0-2.0 $\mu\text{g}/\text{kg}/\text{min}$ has an inotropic effect and improves renal function, while high doses (5.0-10.0 $\mu\text{g}/\text{kg}/\text{min}$) have simultaneous inotropic and vasoconstrictive effects [7, 9].

Cardiorenal syndrome type 2

Cardiorenal syndrome type 2 (chronic cardiorenal syndrome) is defined as a progressive chronic renal impairment due to the chronic impairment of heart function (chronic congestive heart failure) [1-5]. In chronic heart failure, impaired cardiac output and hypoxia cause excessive sympathetic activity, renin-angiotensin-aldosterone system (RAAS) activity, vasopressin system activity, increased oxidative stress in the kidneys, and a disturbance of the L-arginine/NO system in the endothelium of the kidneys. Activation of RAAS increases the levels of angiotensin-2, aldosterone and endothelin-1 in the kidney, which all cause fibrotic processes in the glomeruli and tubulointerstitium and development of end stage chronic renal failure [1-6]. Chronic heart failure also causes anaemia and microinflammation [increased deliberation of cytokine: TNF α (tumour necrosis factor α), interleukin-1, interleukin-6], which additionally contributes to fibrosis of the renal parenchyma and more rapid impairment of renal function [1-6].



Figure 1. Mechanism by which negative sodium and water balance may improve myocardial and renal function in CHF



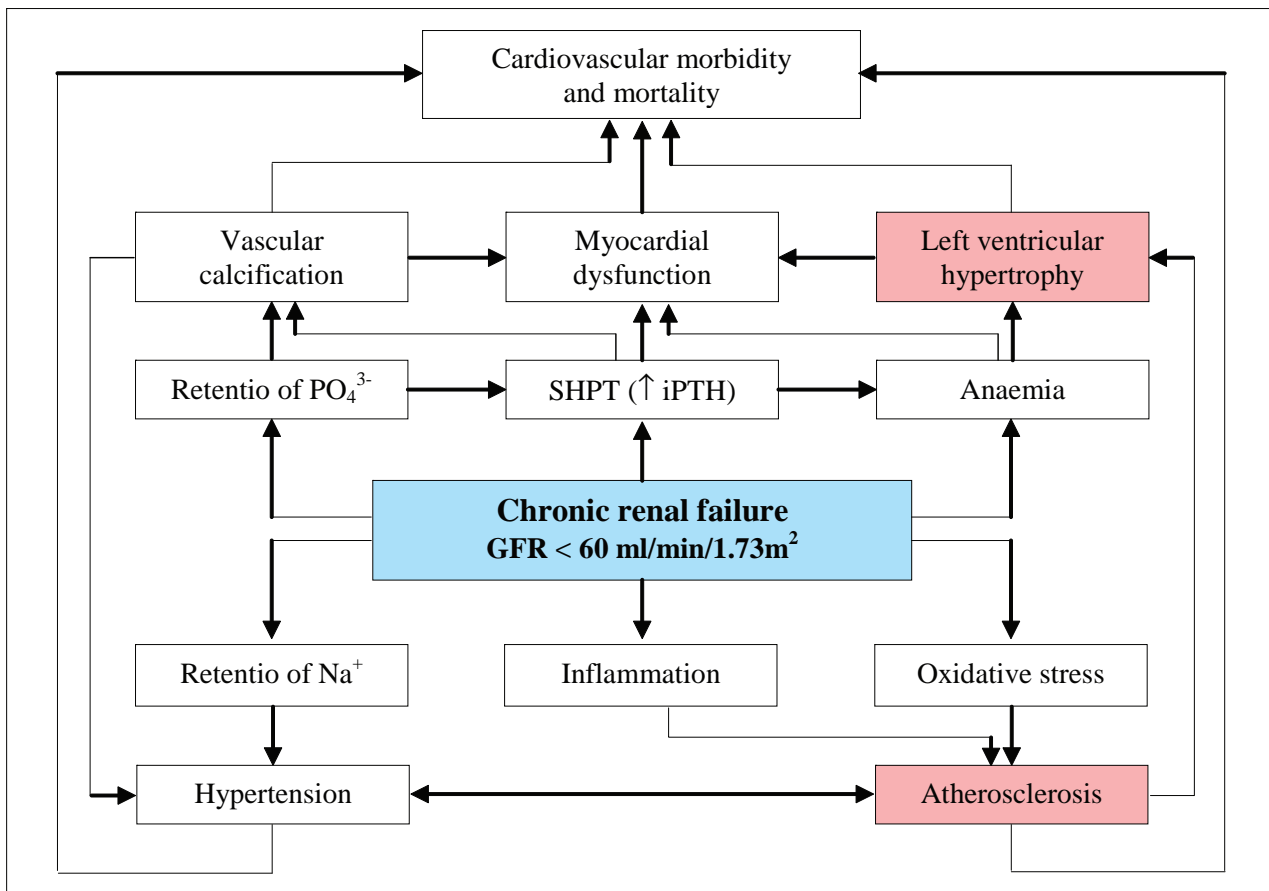
Modified according to reference [13].
CHF - Congestive Heart Failure

The basic goal of cardiorenal syndrome type 2 treatment is improvement of deteriorated heart function, which is main cause of hemodynamic instability, and better renal perfusion (Figure 1) [9, 10]. However, treatment of chronic heart failure may actually worsen renal function. Increased diuresis, blockage of renin-angiotensin-aldosterone system and hypotension exaggerated by medical treatment may all contribute to the worsening of renal function [1-6]. Treatment of patients with chronic heart failure, chronic renal diseases and anaemia with recombinant human erythropoietin improves heart function, leads to regression of left ventricle enlargement and slowing the progression of chronic renal failure [1-6].

Cardiorenal syndrome type 3

Cardiorenal syndrome type 3 (acute reno-cardial syndrome) is defined as an acute damage of heart function (heart failure, arrhythmia, pulmonary oedema) as the result of acute renal damage (acute renal ischaemia or acute glomerulonephritis) [1-6]. Cardiorenal syndrome type 3 is much less frequent than CRS type 1. Due to a sudden impairment in the strength of glomerular filtration and the development of oligoanuria (retention of sodium with

Figure 2. Risk factors for development of cardiovascular complications in patients with chronic renal failure



Modified according to reference [13].
SHPT - secondary hyperparathyroidism, iPTH - intact parathormone, GFR - glomerular filtration rate



acute renal failure [1, 8, 27]. The strategy for preventing acute renal damage in septic patients includes adequate hydration (central venous pressure - CVP 8-12 mmHg, urine output ≥ 0.5 ml/kg/h), maintenance of mean arterial blood pressure (mean arterial blood pressure - MAP ≥ 65 mmHg) and the avoidance of nephrotoxic drugs [28, 29].

Therapy with vasopressors is indicated in patients with hypotension (SAP < 65 mmHg), complete fluid restitution, normal or enlarged cardiac index - SI and absent peripheral vasoconstriction during physical examination [28, 29]. Vasopressors should not be used in patients with a low cardiac index (without inotropics and monitoring), in patients with vasoconstrictive status or those with volume depletion. The most frequently used vasopressin is norepinephrine, also known as noradrenaline. The initial dose used is 0.1 - 0.2 $\mu\text{g}/\text{kg}/\text{min}$, while the upper treatment value is 1.0 $\mu\text{g}/\text{kg}/\text{min}$ [28, 29]. Inotropic therapy (dobutamine) is used in patients with low cardiac index or in patients with lowered cardiac index after the use of, and those with previous fulfilment of intravascular volume (those who reached target restitution). Dobutamine titration begins with a dose of 2.5 $\mu\text{g}/\text{kg}/\text{min}$ and should be increased by 2.5 $\mu\text{g}/\text{kg}/\text{min}$ every 15-20 minutes until normal heart function is maintained (cardiac index - SI = 2.5 - 4.5 l/min/m²) [28, 29].

CONCLUSION

The link between the heart and kidneys is more complex than of a simple pump and filter, and knowing more about the complex interactions between these two organs enables the development of adequate therapeutic strategies for preserving heart and renal function [30-34].

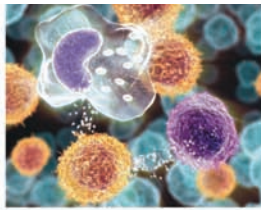
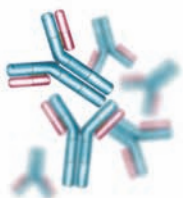
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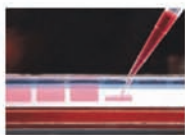


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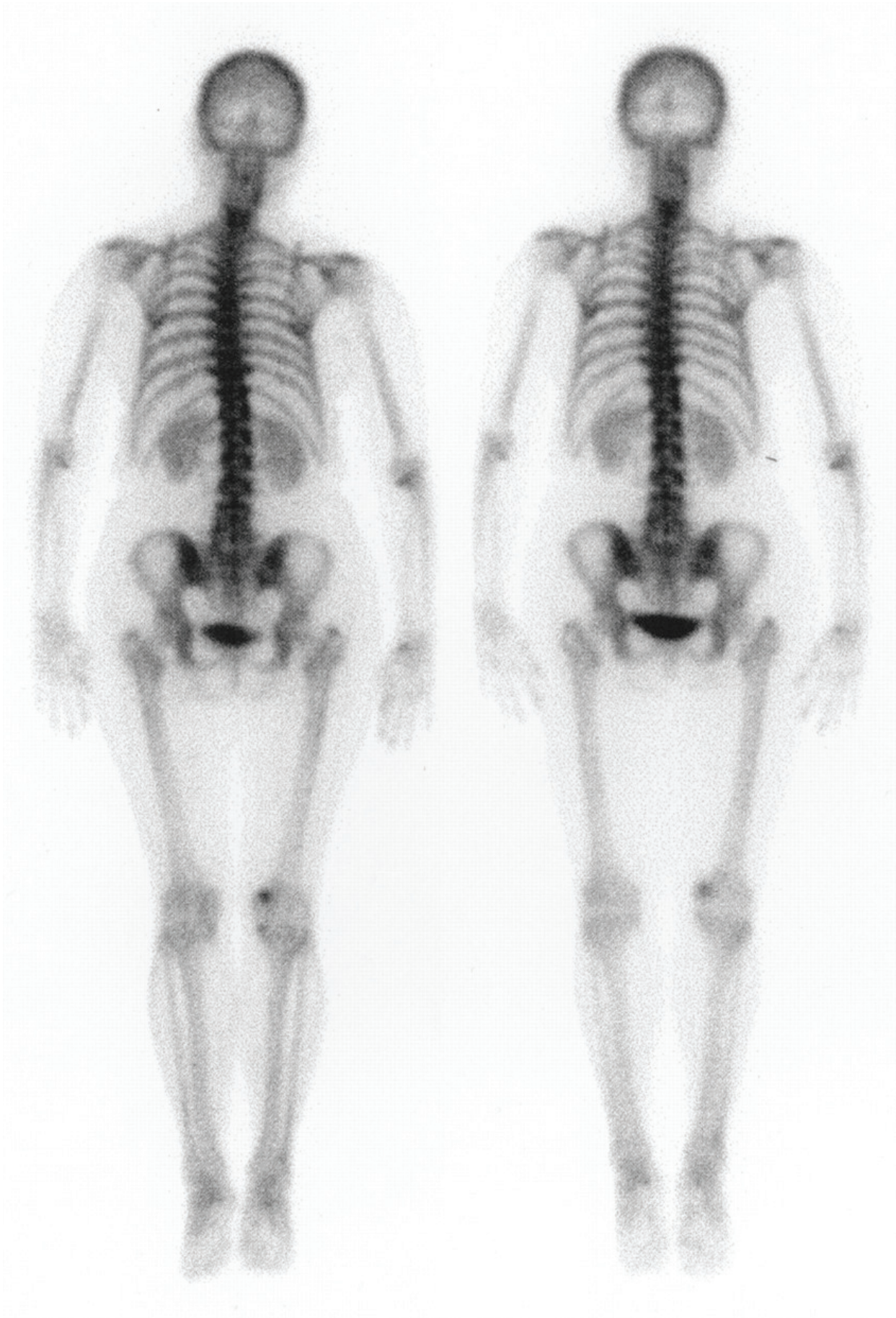
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BASIC PRINCIPLES OF SCINTILLATION DETECTORS AND GAMMA CAMERA

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OSNOVNI PRINCIPI SCINTILACIONIH DETEKTORA I GAMA KAMERE

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ABSTRACT

General principles of construction and the use of the scintillation detectors and gamma camera are presented in this article. Various types of the scintillators and their usage in these devices are also described. The given information is oriented to those medical personnel and medical students, who are neither familiar with physics nor with physical terminology. We hope that our explanations will make them easier to understand aforementioned principles.

Key words: Nuclear Medicine, Imaging, Scintillation detector, Gamma camera

SAŽETAK

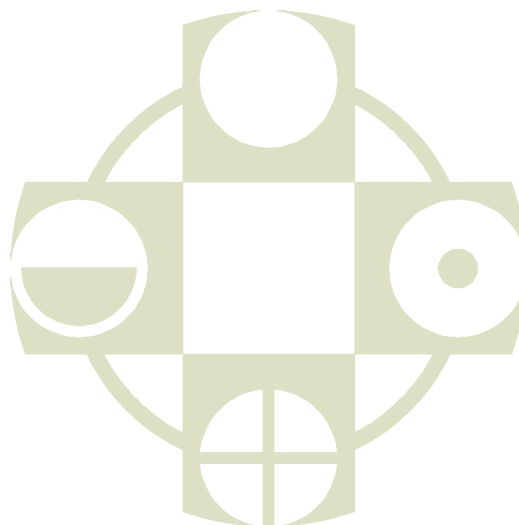
U ovom radu su prikazani generalni principi konstrukcije i korišćenja scintilacionih detektora i gama kamera. Takođe su prikazani različiti tipovi scintilatora i njihovog korišćenja u takvim uređajima. Ove informacije su orijentisane ka medicinskom personalu i studentima, koji nisu dovoljno familijarni sa fizikom i sa fizičkom terminologijom. Nadamo se da će naša objašnjenja njima olakšati razumevanje ovih principa.

Ključne reči: Nuclerna medicina, Snimanje, Scintilacioni detektor, Gama kamera

INTRODUCTION

The discovery of artificial radioactivity in 1934 by the Curies, the development of the scintillation detector in 1948 by Hofstadter, and the construction of a gamma scintillation camera in 1957 by Anger, were key events in the last century in regards to nuclear medicine instrumentation. We intend to describe the

principles of construction and operation of scintillation detectors and gamma cameras. This paper is primarily written to help medical personnel and medical students, who are neither familiar with the physics nor with the physical terminology to be proficient in detector fundamentals.



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SCINTILLATION DETECTORS

Scintillators

Scintillating materials, which can be organic or inorganic, have the property that when excited by ionizing radiation, a gamma photon for instance, will reemit the absorbed energy. The phenomenon is presented as a visible light photons emission following the absorption of gamma photons in the crystal lattice of the scintillator. The mechanism of scintillation is based on the fact that some impurities or imperfections in the crystal lattice can create energy states in the forbidden band, known as activator sites.

What is a scintillation detector?

The scintillation detector constitutes one of the most useful tools available for the detection of a wide range of radiation. The principle of scintillation detector is the interaction of the incident radiation with a scintillating material that releases the energy deposited in the form of light photons. These light photons are subsequently detected by the photomultiplier tubes (PMT), which convert the light photons to electrons. These electrons are multiplied within the PMT to produce a measurable current pulse that is proportional to the energy of the incident radiation.

In nuclear medicine, most detectors are based on inorganic scintillators. These types of the scintillators could be divided into three different groups. The first class of scintillators are those activated by doped impurities; activator sites are produced by adding impurities to the crystal lattice, such as thallium activated sodium iodide (NaI(Tl)), thallium activated cesium iodide (CsI(Tl)), and cerium activated gadolinium orthosilicate ($Gd_2SiO_5(Ce)$). The second type of detectors are made of self-activated scintillating materials, in which case the activator sites are produced by a stoichiometric excess of one of the constituents of the solid. such as cadmium sulphide (CdS) with excess Cd and bismuth germanate (BGO). Finally, the third group of scintillators are those scintillators comprised of pure crystals, in which case activator sites are produced by imperfections in the crystal lattice, such as in diamond.

Properties required for good scintillator

- The detection efficiency should be high for the incident radiation; this corresponds to the material having a high atomic number and high density.
- The crystal itself must be transparent to the scintillation light.
- The conversion of radiation into light should be linear over a wide range of energies. The light yield should be proportional to the deposited energy.
- The crystal should have high stopping power, which depends on the effective Z (or high linear attenuation coefficient).
- The crystal should convert the energy of the radiation absorbed into detectable light with high scintillation efficiency, i.e., high light output (LO). It is the number of visible

photons produced in the scintillator under gamma radiation. This is usually is expressed in terms of photons/MeV.

- The decay time of the produced scintillation light should be short.
- The index of refraction of a scintillator should be similar to that of glass (1.5), and the wavelength of scintillation light should be similar to the maximum PMT sensitivity.
- The scintillator should be both available and inexpensive.

These properties influence energy resolution, which defines the ability of the detector to determine the incident energy of the radiation. High resolution is necessary in order to distinguish gamma-sources of slightly different energies.

Thallium doped sodium iodide NaI(Tl) is the most widely used scintillation material. Its light output is greater than that of other scintillators, and it has a convenient emission range (in coincidence with maximum efficiency region of photomultiplier with alkali photocathodes). The main disadvantage to NaI(Tl) is hygroscopy.

Cesium iodide doped with sodium CsI(Na) is currently a widely used material. High light output (85% of that of NaI(Tl)), emission in the blue spectral region (coincident with the maximum sensitivity range of the most popular PMT with alkali photocathodes), and substantially lower hygroscopicity in comparison with that of NaI(Tl), makes this material a good alternative for NaI(Tl) in many standard applications.

Complex oxide crystals, such as gadolinium silicate doped with cerium ($Gd_2SiO_5(Ce)$ or GSO), BGO, CWO, PWO, and NBWO, have a number of advantages over alkali halide crystals: high effective atomic number, high density, good energy resolution, low afterglow, and non-hygroscopy. Due to these features, detectors with oxide crystals are fail-safe, and there is no need for hermetisation.

Zinc selenide ZnSe(Te) scintillation material was created especially for matching with the photodiode, with an emission maximum at 640 nm. However, ZnSe(Te) crystals possess poor transparency.

Organic scintillators can be either liquid or solid and are characterised by their high response speed and relatively low light output.

Construction and Function of Scintillation Detectors

The basic scintillation detector consists of

- Scintillator (usually crystal NaI(Tl))
- Light guide, which is usually a thin layer of some transparent material with appropriate index of refraction, similar to the of refraction index of the crystal and photocathode
- Photo-detector (usually photomultiplier tube-PMT)

The Photomultiplier tube (PMT) consists of

- Vacuum glass envelope with a transparent window to couple the scintillator with a light guide
- Photocathode to absorb scintillation photons and to emit photoelectrons

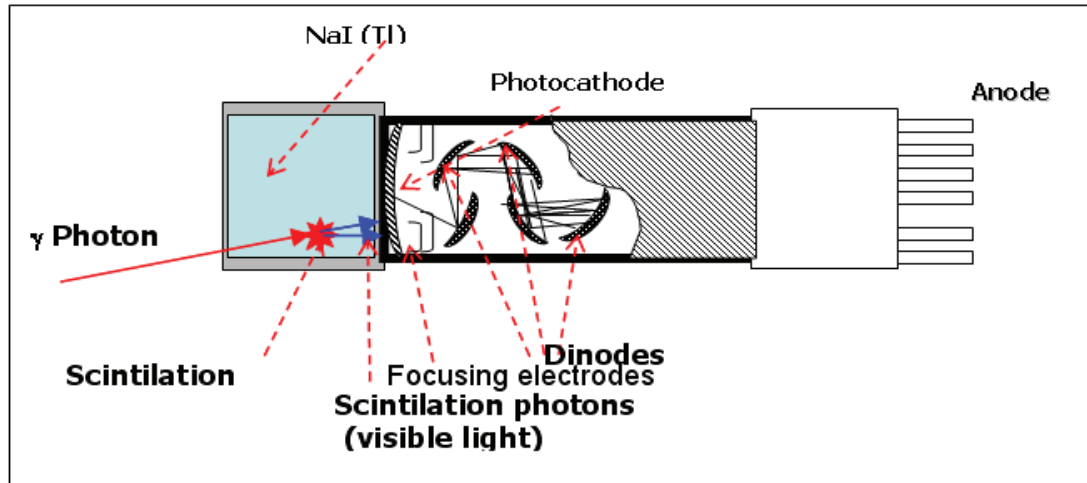


Figure 1. Schematic of a scintillator detector.

GAMMA SCINTILLATION CAMERA

- Focusing electrodes to guide photoelectrons to the first dynode
- Dynode chain - there is a voltage drop between each dynode (100-300 V)
- Anode - the output stage of the PMT
- * Amplitude of PMT anode output pulse is proportional to the energy E_γ of the incident photon. This is a very important characteristic of scintillation detector.

How does it work?

A fraction of the light photons produced in the scintillator will hit the PMT glass window. The light that passes through the glass envelope will hit the photocathode. Commonly, a photocathode consists of a mixture of potassium, cesium, sodium, and antimony. The photocathode must be thick enough to absorb the light photons, yet thin enough to prevent absorption of the photoelectron. Its purpose is to absorb the photon and emit an electron. Focusing electrodes guide the electrons, or the photoelectrons, to the first dynode. When the photoelectron strikes the dynode after being accelerated through 100-300 V, it will cause an emission of 2-5 secondary electrons, which in turn will be attracted to the next dynode and produce a multiplication of the 2-5/dynode stage.

The multiplication factor for a dynode is given by

$$\delta = \frac{N_s}{N_p}$$

where N_s is the number of secondary photoelectrons and N_p is the number of incident primary electrons. Typical values of δ are approximately between 4 and 5, and the overall gain in the PMT is given by

$$\text{gain} = \alpha \delta^N$$

In this relationship, α is the collection efficiency (~ 1) of the photoelectrons and N is the number of dynodes.

The gain of a 10-stage PMT tube is $\sim 5^{10}$ or 10^7 .

While the shape and design of the detector and electronic processing have been altered since its inception, the basic components and principles of the gamma camera have changed little over that time.

Hal Anger 1958 developed a visualisation device with 10-cm diameter circular NaI(Tl) crystal, pinhole collimator, and 7 PMTs. The author named this device "scintillation camera", although it is now usually referred as an "Anger camera".

What is Nuclear Imaging?

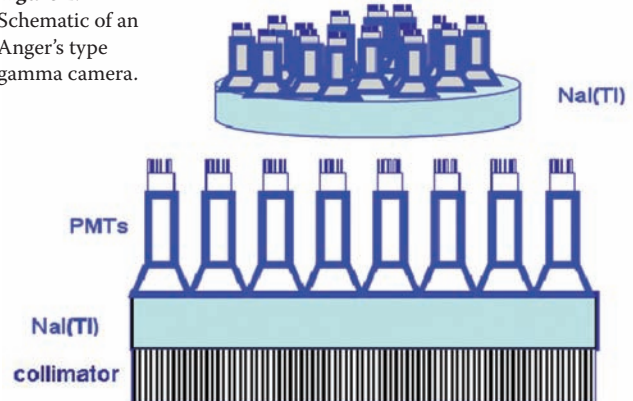
Nuclear imaging involves injecting a small amount of a chemical substance, tagged with a short-lived radioactive tracer, into a patient. Depending on the chemical substance used, the radiopharmaceutical concentrates in the part of the body being investigated and emits gamma rays. A gamma camera then detects the source of the radiation to build a picture. These are called scans.

Nuclear medical imaging may be divided into three categories:

- Conventional or planar medical imaging
- Single photon emission computed tomography (SPECT)
- Positron emission tomography (PET)

Conventional and SPECT imaging use gamma cameras of the Anger type, which are more or less modified. Cameras of this general type have a single crystal viewed by arrays of detectors (Figure 2).

Figure 2. Schematic of an Anger's type gamma camera.



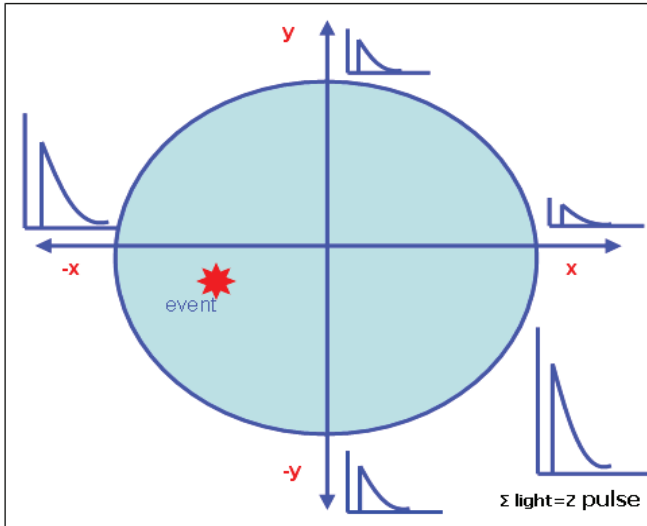


Figure 3. Schematic of detection and position of the event in the crystal of Anger's gamma camera.

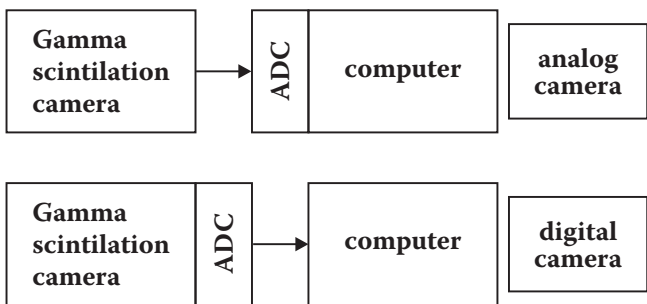


FIGURE 4. Analog and digital type GSC.

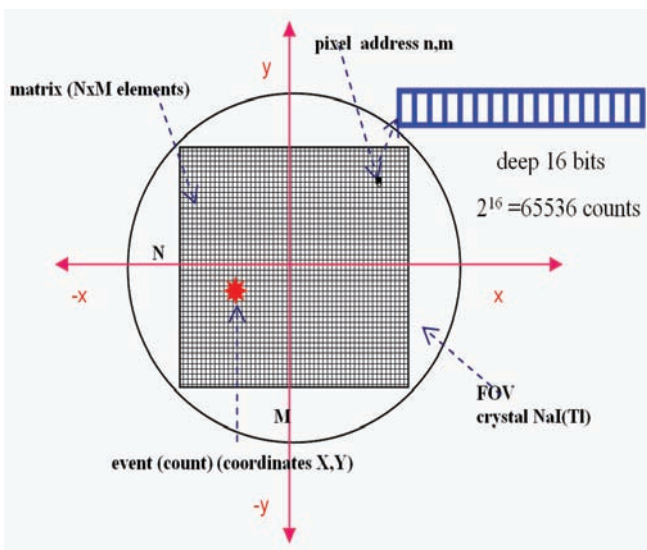


Figure 5. Computer memory and principle of data storage.

How do Gamma Scintillation Cameras work?

The emitted photons are first discretised by direction using a collimator. The collimator ensures that each small part of the crystal views only a small area of the organ to be imaged. Each interactions of a gamma ray with the detector crystal is called an 'event'. Scintillation imaging produces many single events in the crystal (Figure 3). The scintillation, which is produced in the crystal, originates very close to the point of interaction (event).

Each photomultiplier tube in the array detects the scintillation light of each single event. The PMT converts the detected light into an electronic pulse. The amplitude pulse is proportional to the intensity of the detected light; i.e., it is related to the proximity of the PMT to the point of interaction (Figure 5). Each event is located using information collected from each PMT. From all detected outputs, a position-specific logic circuit (Anger logic) estimates the position of each event. Because of this processing, the vertical and axial positions of PMTs are weighted by their electric signal responses from all PMTs. That circuit then gets X and Y coordinates of each single event. A single image is comprised of many events, represented as dots on the analog image.

Gamma Camera Computer System

For optimal performance, the gamma scintillation camera is often coupled with a dedicated computer. Analog-to-digital conversion of the signal from gamma camera is provided by an analog-digital converter (ADC) module. This module is part of dedicated computer in older models of analog gamma cameras, or part of gamma camera itself in newer digital models (Figure 4).

Signals for the definition of horizontal and vertical positions (X and Y coordinates, respectively) of the events will then be converted to digital form. The ADC conversion is usually performed with ADC converters for each channel (separately for the X and Y coordinate). As trigger for AD conversion process of each single event uses corresponding Z pulse. The ADC module and dedicated software developed in our institution (9) could be an example how it can be realised.

The digital image in the computer's video memory matrix is presented as a rectangular object in (or around) the field of view of detector crystal (FOV), which consists of NxM elements (Figure 5). These elements are usually called pixels. In most cases, N is equal to M and the shape of the matrix is a rectangular quadrilateral. Each pixel of the matrix has its own unique address (n,m), which corresponds to the X and Y coordinates of the event in the crystal. According to the given digitalised X and Y coordinates of the actual event, an appropriate pixel is increased by 1 for each registered event. In this case, an event is commonly referred to as a count. Many single events will be represented in video memory as a digital image of the spa-



tial distribution of the radiopharmaceutical. The number of events that can be stored in each pixel depends on the third dimension (depth) of the used matrix. A matrix of n bits deep can store 2^n events. It is common practice in nuclear medical imaging to use matrices 8 to 64 bits deep and with up to 512x512 pixels. Figure 6 presents one common nuclear medicine image, a scintigram of the thyroid.

Computer systems coupled with gamma camera enable data processing, safe long-term data storage, provide dynamic, gated and ECT studies. If an image in computer memory is digital, we could further process using, for example, digital filtration, edge detection and enhancement, shape recognition, etc.

Newer trends in construction

Over the past decade, great progress has been made in combining anatomical imaging, such as CT or MRI, with modes of functional imaging, such as SPECT and PET. The first advancement was the development of software for the fusion of images generated from those different "stand alone" machines, followed by the development of hybrid devices such as SPECT/CT and PET/CT. There are currently many models of hybrid imaging devices, with SPECT or PET as functional imaging devices that are often coupled to a 64-slice CT for use as anatomical imaging devices. More powerful hybrid imaging machines such as PET/MRI are being developed as the modality of choice in clinical imaging, and such machines will likely be the ultimate future imaging modality.

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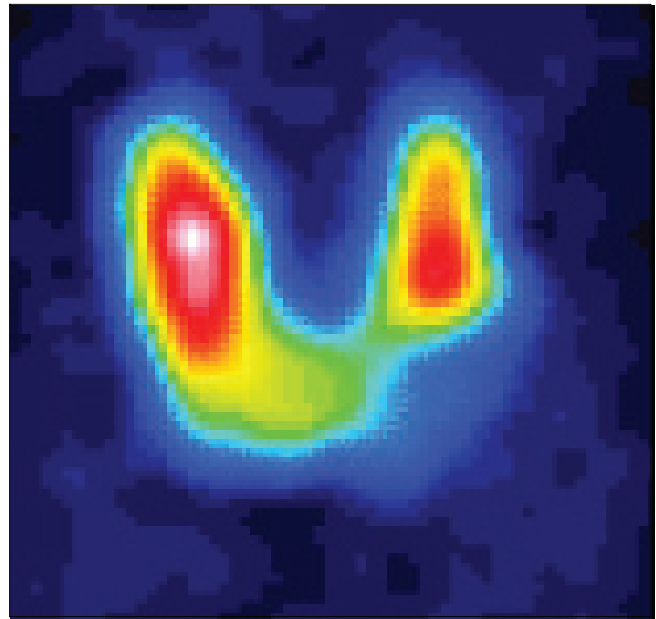


Figure 6. Image of the thyroid ("cold node" in left lobe).

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ADENOMYOEPITHELIOMA OF THE BREAST AS A DIAGNOSTIC PROBLEM

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ADENOMIOEPITELIOM DOJKE KAO DIJAGNOSTIČKI PROBLEM

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ABSTRACT

Adenomyoepitheliomas are extremely rare breast tumours that were not described and classified until after the 1990s. They are characterised by biphasic proliferation of ductal and myoepithelial cells organised in ribbon-like, solid, tubular or lobular arrangements. During years adenomyoepithelioma represent poorly understandable diagnostic entity which biological behaviour cannot be always predicted on the basis of the cytological characteristics and histological architecture. Diagnosis by clinical examination and imaging studies remains difficult, and frozen sections of selected areas can potentially be misdiagnosed as malignant.

In this study, we present a case of a breast adenomyoepithelioma in 68-year-old woman. The diagnosis was made with immunohistochemistry following a surgical excision.

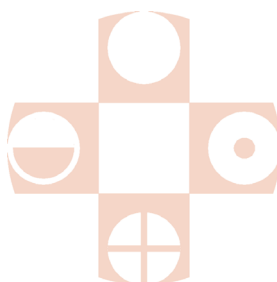
Keywords: breast tumours, adenomyoepithelioma, immunohistochemistry.

SAŽETAK

Adenomioepiteliomi su retki tumori dojke, detaljno opisani i klasifikovani tek devedesettih godina prošlog veka. Karakteriše ih bifazna ćelijska proliferacija duktalnih i mioepitalnih ćelija organizovanih u trakaste, solidne, tubularne ili lobularne aranžmane. Godinama predstavljaju slabo razumljiv dijagnostički entitet, čije se biološko ponašanje ne može uvek predvideti na osnovu citoloških i histološko-arhitekturnih osobina. Postavljanje precizne dijagnoze, na osnovu kliničkog nalaza i radioloških-imidžing metoda, je veoma teško, a ex tempore analiza pojedinih tumorskih zona može biti pogrešno interpretirana kao maligna.

U ovom radu, prezentujemo slučaj adenomioepitelioma dojke kod žene stare 68 godina, koji je nakon hirurške ekscizije, dijagnostikovao određivanjem imunofenotipa ćelijske populacije ovog tumora.

Ključne reči: tumori dojke, adenomioepiteliom, imunohistohemija.



ABBREVIATIONS

*H&E - haematoxylin and eosin;
LMW CK - low-molecular weight cytokeratin;
HMW CK high-molecular weight cytokeratin;
 α -SMA - smooth muscle actin;
GFAP - glial fibrillary acidic protein;
ER - estrogen receptor; PR - progesteron receptor.*

SKRAĆENICE

*H&E - hematoksilin i eozin
LMWCK - nisko-molekularni citokeratin
HMWCK - visoko-molekularni citokreatin
 α -SMA - glatko-mišićni aktin
GFAP - glijalni fibrilarni protein
ER - estrogen receptor PR- progesteron receptor*

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INTRODUCTION

Adenomyoepitheliomas are extremely rare breast tumours. Hamperl (1970) was the first to describe this tumour¹, and in 1991, Tavassoli classified it as a form of breast neoplasia². Adenomyoepitheliomas are unique tumours formed of epithelial and myoepithelial cells, and other structures are sometimes present, as well. Classification is occasionally further complicated by papillary proliferation or microglandular adenosis within the foci of the tumour mass.

These tumours are most commonly restricted in growth and benign in character. However, sometimes their biological behaviour is unpredictable as assessed by macroscopic, histological and immunohistochemical features.

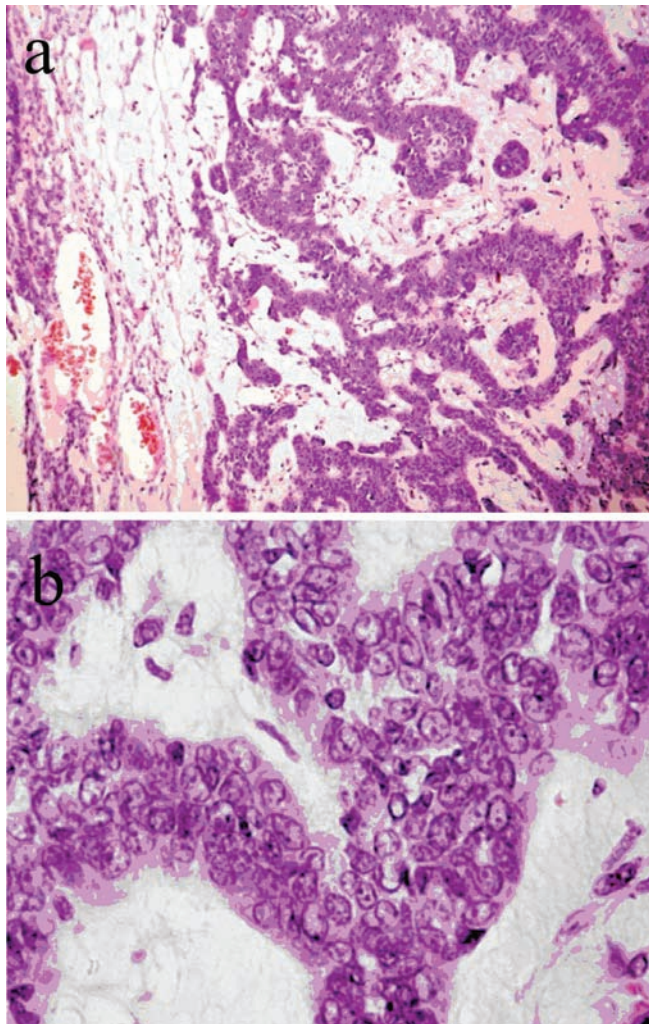


Figure 1: Histological features of adenomyoepithelioma (H&E staining). a) The well-restricted tumour is composed of proliferated ductal epithelial cells, myoepithelial cells and myxoid stroma (original magnification, x100). b) The tumour cells demonstrate a slight polymorphic morphology, with pale eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli (original magnification, x400).

CASE REPORT

A 68-year-old woman was admitted by her family doctor because of a change in the upper outer quadrant of the left breast that was first noticed a few months ago. Planar X-ray mammography was suspicious for neoplasia, but ultrasound results were nonspecific. Clinical examination revealed the presence of a subcutaneous, localised, solid, mobile mass of approximately 4 cm diameter, nontender to palpation. Axillary lymphadenopathy was absent. A multidisciplinary oncology team recommended surgical treatment. A biopsy of the mass was performed, and an intraoperative ex tempore histological analysis was done.

Macroscopically, the specimen was a round, solid, well-circumscribed, irregular mass 35×25×20 mm in diameter. Tissue sections revealed a white-yellow interior. The findings of the microscopic ex tempore analysis were nonspecific, as the frozen section showed microscopic structures with both benign and malignant features, varying by tissue area.

The samples were fixed in 4% formaldehyde solution and embedded in paraffin. Five-µm-thick tissue sections were stained with hematoxylin and eosin (H&E).

On further microscopic exam, the tumour was well-encapsulated, but in several foci the tumour appeared to invade the surrounding tissue in tiny bands. The tumour consisted of myxoid stroma and cells of slightly polymorphic morphology with a myoepithelial-like shape that formed anastomosis-like bands, ranging in width from one to more than 10 cells (figure 1a). The cytoplasm was pale, nuclei were large and vesicular and nucleoli were prominent (figure 1b). Inside the bands and occasionally outside of them, foci of pseudo-luminal glandular structures suggesting ductal differentiation of the tumour were noted. Within those foci, the cells had lightly eosinophilic cytoplasm and hyperchromic nuclei. There were 5 mitotic figures per 10 areas of large microscopic magnification. Because of suspicion for double histological differentiation, additional immunohistochemical analyses were performed.

On immunohistochemical analysis, tumour cells demonstrated a diffuse, moderate-to-strong positivity for vimentine, low-molecular weight and high-molecular weight cytokeratin (LMW and HMW CK, respectively) (figures 2a, c, and d). They also displayed sporadic positivity for S-100 protein, smooth muscle actin (α-SMA), and glial fibrillary acid protein (GFAP) (figures 3a, b, and c). About 8% of the nuclei of tumour cells stained positive for Ki-67 (figure 3d). Staining for desmin, HER-2, estrogen (ER) and progesterone (PR) receptors was negative.

Based on this microscopic analysis and immunophenotype, a diagnosis of benign adenomyoepithelioma was established. The patient was discharged and advised to follow up regularly. The patient remained well throughout two years of follow-up after the surgery, with no apparent recurrence.

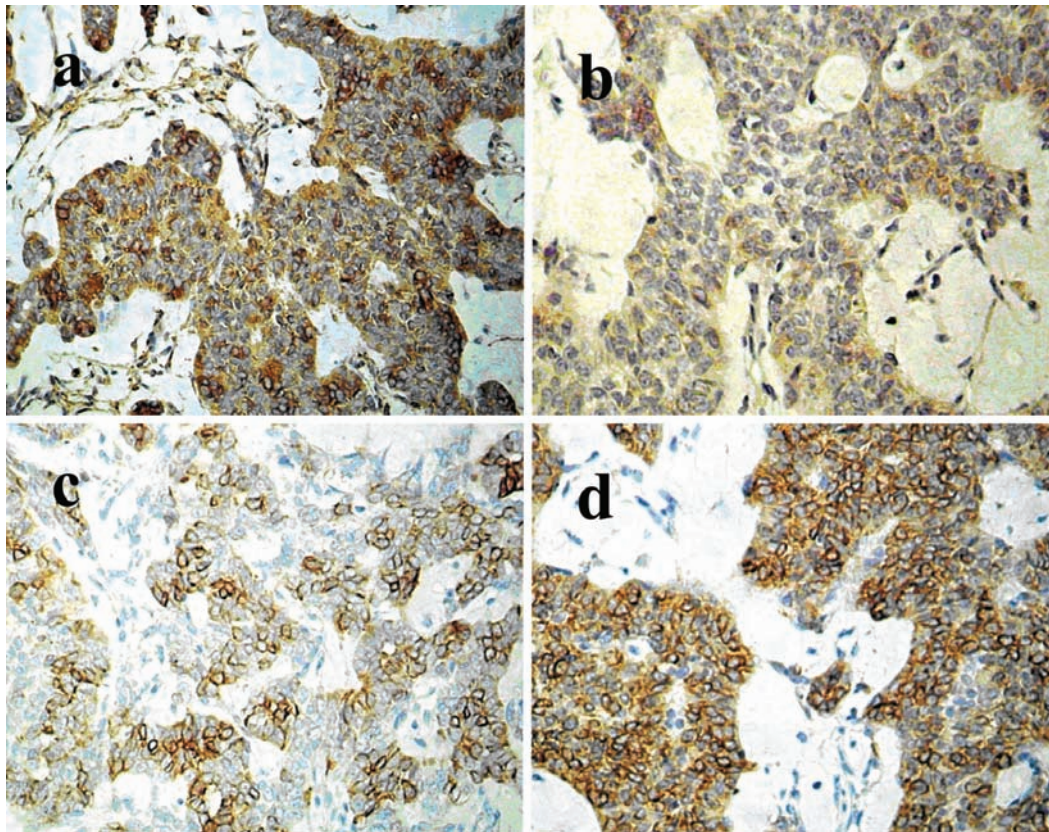


Figure 2: Immunohistochemical staining (original magnification, x200).
 a) The tumour cells demonstrate moderate to strong expression of vimentin, and
 b) discrete and weak expression of desmin.
 c) Approximately 50% of tumour cells are strongly positive for CK HMW.
 d) Diffuse, strong staining for CK LMW.

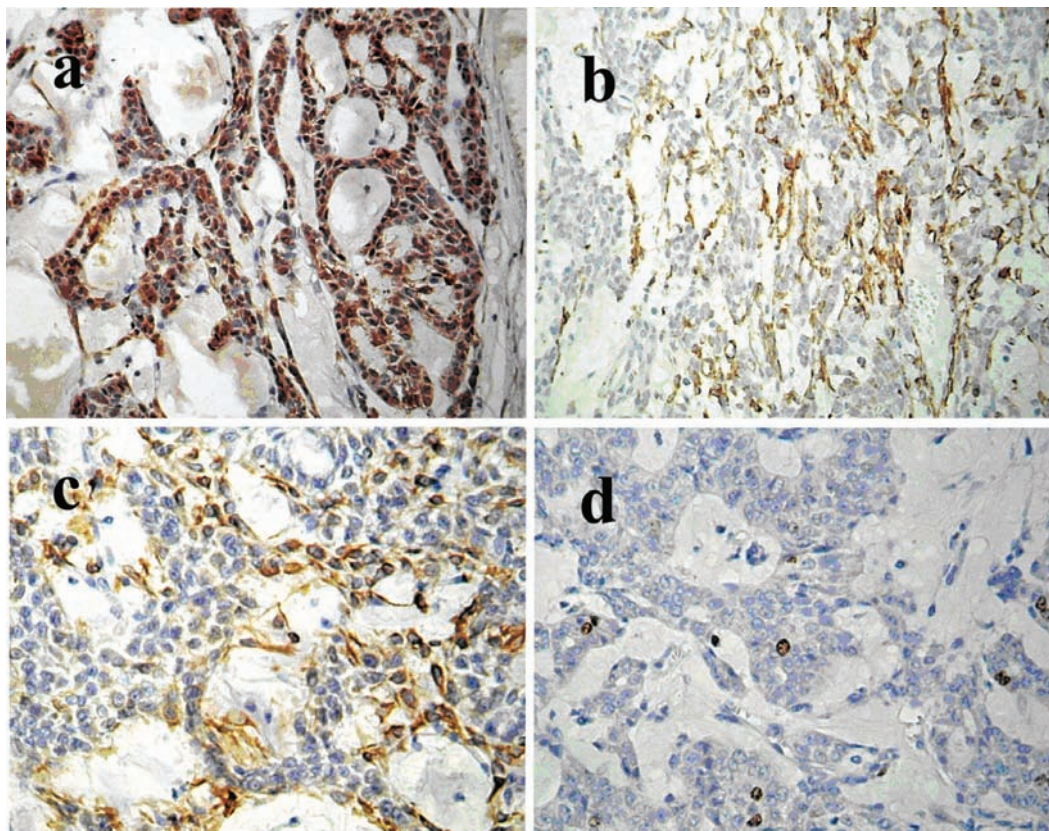


Figure 3: Immunohistochemical staining (original magnification, x200).
 a) The tumour cells demonstrate moderate to strong expression of S-100 protein.
 b) Myoepithelial cells positive for α -SMA and
 c) GFAP.
 d) The nuclei of the tumour cells show focal positivity for Ki67 (Ki67 index = 8%).



DISCUSSION

Breast adenomyoepitheliomas are extremely rare, so data about these tumours are limited to individual case-reports.³⁻¹³ They appear to occur in a wide age range, from 26 to 82 years of age. In the majority of the patients, the mass's initial presentation was as a solid, clearly circumscribed, painless lesion, localised in a peripheral quadrant of the breast. The usual tumour size is from 0.6-17 cm, with the largest mass described in a 30-year-old woman.¹³ They more frequently appear as solitary rather than multifocal masses.⁸ Reported colors include light brown, grey, white or yellow. X-ray mammography and ultrasound imaging often suggest malignancy.^{4,7}

The specific histological presentation depends on the presence of glandular-epithelial and myoepithelial proliferation, the degree of spindle-cell differentiation and the extent of fibrosis. Thus, adenomyoepitheliomas appear closely related to ductal adenomas¹⁴ and pleomorphic adenomas.¹⁵ However, these tumours are more commonly found in subareolar regions rather than in the lateral breast quadrants, and they probably originate from large lactiferous ducts.^{6,15}

The most common microscopic appearance of adenomyoepithelioma is tubular with glandular structure proliferation, though myoepithelial differentiation can be found in peripheral islands of solid tumour or in the basal cells of tubular structures. Rarely, myoepithelial proliferation appears as wide bands and trabeculae that are both separated by fibrovascular stromal cells. Epithelial and myoepithelial cells are incorporated into abundant mixed stroma¹² that sometimes contain areas of calcification, cystic degeneration, and cartilaginous and bony metaplasia. In our case, myoepithelial cells formed anastomosing bands of one to many cells thick, with additional foci of ductal differentiation. Abundant mixed stroma was found in the area of this epithelial "building block."

Cytological atypia and mitotic activity were rare or absent in the lesions where myoepithelial cells were seen to have a polygonal configuration or a clear papillary form. These cells were often found in the lesions that appeared to be growing in spindle-shaped formations. They were noted to contain increases in mitosis, nuclear polymorphism, and hyperchromasia, and rare multinuclear cells were seen in them. However, cytological atypia is not a valid criterion for diagnosing adenomyoepithelioma, because some tumours without that feature progressed aggressively.^{2,7} Therefore, in the absence of an obviously malignant pattern of cytomorphology, invasive development remains the only reliable criterion for establishing a diagnosis of malignancy.

The immunohistochemical analysis of cell phenotype, and thus the establishment of a diagnosis, is greatly aided when the pathologist is familiar with the great morphological diversity of adenomyoepitheliomas. Normal myoepithelial cells strongly express LMW CK, α -SMA, myosin, S-100, CD-10 and maspin.⁸ Because S-100 protein

is expressed in both glandular and myoepithelial cells, it cannot be used as diagnostically specific tumour marker for adenomyoepithelioma.¹¹ Epithelial cells regularly stain positive for cytokeratin and carcinoembryonic antigens.^{5,6} Both cell types are variably positive in response to vimentine and immunonegative staining for epithelial membrane antigen, p53 and HER-2.⁸ Also, the response to staining for ER and PR receptors is variable; if they are immunohistochemically present, they are restricted to the nuclei of epithelial cells.^{7,9} In our case, most tumour cells were positive for vimentine, LMW and HMW CK, and a smaller number were positive for S-100 protein, GFAP and α -SMA. Ki-67 was visualised on 8% of tumour cells. In the available literature data about mitotic indices, data about the association between number of mitotic figures and tendency towards malignancy are scarce. One paper reported a series in which 7 per 10 areas of large microscopic magnification were considered a diagnostic sign suggesting malignancy.¹⁶

Adenomyoepitheliomas are most commonly benign, well-circumscribed, and encapsulated tumours that are most frequently treated with surgical excision.⁷ However, although they are enclosed within a pseudo-capsule of connective tissue, masses often infiltrate their surrounding structures.¹⁰ Due to this invasive tendency, the natural course of these lesions is ultimately unpredictable. Local recurrence usually occurs two years after initial excision.² In some cases, multiple recurrences were reported, likely resulting from incomplete tumour excision.^{3,7} Mastectomy, radiotherapy and axillary dissection for benign adenomyoepithelioma should be avoided, except in the case of frequently recurrent lesions or tumours with malignant characteristics.¹⁶⁻¹⁹

The reports concerning malignant adenomyoepithelioma are rare. Neoplasm can be detected incidentally as a separated mass or after an excision of the adenomyoepithelioma.² Histological characteristics include increased mitotic activity, necrosis, cellular polymorphism, and, frequently, invasion of tissue surrounding the tumour. Malignant alteration could be limited to the epithelial or myoepithelial zones, or rarely to both of them. The location of malignant zones might alternate within the same tumour, e.g., the foci of benign characteristics and obviously malignant lesions could be bounded by the zones with benign features.^{16,17} Some tumours described as malignant adenomyoepitheliomas recurred locally, while other masses metastasised, resulting in fatal outcomes.¹⁶⁻¹⁹

CONCLUSION

Breast adenomyoepithelioma is usually a well-circumscribed tumour; as such, complete surgical excision of the mass remains the preferred therapeutic strategy. The definitive diagnosis is established with vigilant microscopic analysis and immunohistochemical staining. This approach avoids an incorrect pathological diagnosis that could lead to an unnecessary procedure such as a major surgery.



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