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Heart transplant rejection pathology

Patologija odbacivanja transplantata srca

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Abstract

Background/Aim. Heart transplantation is the most effective way to treat patients in the terminal stage of heart failure. Endomyocardial biopsy has proven to be a safe and appropriate technique, with little sampling error, and remains, to this day, one of the most commonly used methods for diagnosing acute rejection. In 1990, the International Society of Heart and Lung Transplantation defined a standardized system for grading the severity of acute transplant rejection regarding endomyocardial sampling histopathological analysis. The aim of the study was to assess the morphological, immunohistochemical, and immunofluorescent markers of cell- and antibody-mediated rejection of heart transplants in patients monitored during 2020. **Methods.** From 31 patients transplanted at the Clinic for Cardiac Surgery of the University Clinical Center of Serbia, endomyocardial biopsy material was obtained, then processed and analyzed at the Institute of Pathology of the Faculty of Medicine, University of Belgrade. **Results.** The average Transplant Rejection Score (TRS) value was 0.42. The Spearman's correlation test did not show a statistically significant relationship between the TRS value and the difference between the ejection fraction values three and twelve months after transplantation. **Conclusion.** The mean TRS value obtained in this study suggests dominant cell-mediated graft rejection.

Key words:
biopsy; heart; heart function tests; heart transplantation; histological techniques; immunohistochemistry; organ dysfunction scores.

Apstrakt

Uvod/Cilj. Transplantacija srca predstavlja najefikasniji način lečenja bolesnika u terminalnom stadijumu srčane insuficijencije. Endomiokardna biopsija se pokazala kao bezbedna i prikladna tehnika, sa malom greškom pri uzorkovanju i do danas ostaje jedna od najčešće korišćenih metoda za dijagnostiku akutnog odbacivanja transplantata. Internacionalno društvo za transplantaciju srca i pluća je 1990. godine definisalo standardizovani sistem za gradiranje težine akutnog odbacivanja transplantata korišćenjem patohistološke analize uzoraka endomiokarda. Cilj rada bio je da se analiziraju morfološki, imunohistohemijski i imunofluorescentni pokazatelji ćelijama- i antitelima-posredovanog odbacivanja transplantata srca kod bolesnika praćenih tokom 2020. godine. **Metode.** Od 31 bolesnika lečenih na Klinici za kardiohirurgiju Univerzitetskog kliničkog centra Srbije uzet je uzorak endomiokardne biopsije, a zatim obrađen i analiziran na Institutu za patologiju Medicinskog fakulteta Univerziteta u Beogradu. **Rezultati.** Prosečna vrednost stepena odbacivanja transplantata (*Transplant Rejection Score* – TRS) iznosila je 0,42. Spirmanovim testom korelacije nije pokazana statistički značajna veza između vrednosti TRS i razlike vrednosti ejekcione frakcije, 3 i 12 meseci posle transplantacije. **Zaključak.** Prosečna vrednost TRS, dobijena u ovom istraživanju, upućuje na dominantno ćelijama-posredovano odbacivanje transplantata srca.

Ključne reči:
biopsija; srce; srce, funkcijski testovi; transplantacija srca; histološke tehnike; imunohistohemija; skorovi, disfunkcija organa.

Introduction

Heart transplantation is the most effective way to treat patients in the terminal stage of heart failure [1, 2]. The International Society for Heart and Lung Transplantation (ISHLT) has suggested guidance for identifying potential candidates for heart transplantation [1, 3]. Unlike improvement and enhanced final result, transplant rejection still represents the heart transplant's Achilles heel [1–4]. Heart transplant rejection (HTR) can be manifested interoperatively, early or a few years after the

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transplantation [1–6]. In the posthospital discharge period, even a few years, the postoperative HTR time is essential for determining the etiology of transplant rejection and its diagnosis. With the development of heart transplantation, heart endomyocardial biopsies (EMBs) have an important role in diagnosing complications and grade of graft rejection [1]. EMB has been shown to be a safe and appropriate technique, with little sampling error, and remains, to this day, the most commonly used method for diagnosing acute rejection [2]. In 1990, the ISHLT defined a standardized system for acute HTR grading. The aim of this system was to enable grading which was easy, reproducible and could be extrapolated to other systems. Regardless of the grading system, the factors that are assessed are: the nature, intensity, and distribution of infiltrates of inflammatory cells; presence or absence of edema; presence or absence of cardiomyocyte damage [2, 3]. The ISHLT system is defined by four grades: grade 0R (no rejection, inflammatory cell infiltration, and cardiomyocyte damage); grade 1R (mild rejection, interstitial or perivascular inflammatory infiltrate with or without the focus of cardiomyocyte damage); grade 2R (moderate rejection, ≥ 2 foci of infiltration by inflammatory cells with cardiomyocyte damage); grade 3R (severe rejection, diffuse inflammatory infiltrate with multifocal cardiomyocyte damage, edema, vasculitis, and interstitial hemorrhage). Granulation connective tissue with inflammatory cell infiltrate is present at the sites of previous EMBs, and it is necessary to distinguish them from histological indicators of acute rejection [2, 7]. Examples of patients with hemodynamic and echocardiographic indicators of graft dysfunction but without histological evidence of cellular rejection are presented in the literature. Such cases represent humoral or antibody-mediated rejection (AMR), which is histologically presented by immune cell infiltrates, interstitial edema, hemorrhage, damaged capillaries, venules, and arterioles as well as cardiomyocyte necrosis [2, 8, 9]. Based on histological and immunocytochemical characteristics, Hammond et al. [8–10] classified AMR into five degrees: negative (without AMR), ambiguous evidence of AMR (endothelial cell activation, edema, damage with or without hemorrhage, and without inflammation or thrombosis), mild AMR (leukocytoclastic vasculitis), moderate AMR (arteriolitis, interstitial edema, fibrin accumulation) – identical to severe acute graft rejection.

The aim of this study was to examine the pathophysiological parameters of HTR, such as inflammatory cell infiltration, cardiomyocyte damage, damaged capillaries, venules and arterioles, and interstitial edema in patients monitored at the Cardiac Surgery Clinic of the University Clinical Center of Serbia in the period from January to December 2020.

Methods

Patients studied

Biopsy material was obtained from 31 patients treated at the Clinic for Cardiac Surgery of the University Clinical Center of Serbia as a clinical routine scheduled for monitoring transplanted patients. Biopsy samples were taken according to the following scheme: the first five biopsy samples were taken every 15 days from the day of transplantation. The next three biopsy samples were taken monthly, and then for a period of 2 years, an endomyocardial biopsy sample was taken every 3 months. During the third and fourth year after transplantation, samples were taken every four months, while biopsies were not routinely performed from the fifth year after transplantation. Obtained biological material was processed and reviewed at the Institute of Pathology of the Faculty of Medicine, University of Belgrade. The clinical and morphological data analyzed were: gender, age, age at the moment of the transplantation, heart disease diagnosis, the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profile, the New York Heart Association (NYHA) classification, and the ejection fraction (EF). For the purpose of calculating Transplant Rejection Score (TRS) ACR was graded as follows according to the ISHLT: 0R = 0, 1R = 1, 2R = 2, 3R = 3. AMR was absent in all samples from all 31 monitored patients, thus grade for AMR was 0. The TRS was calculated as the quotient of the total number of rejections and the number of biopsies during a year. Since there were no AMR rejection, calculated TRS presented dominant cellular type of rejection.

Histological sample analysis

The EMB sample was submerged in 4% buffered formalin for 12–24 hrs, then rinsed with water and dehydrated in growing concentrations of alcohol (from 70% up to absolute alcohol) over 24 hrs. The samples were lyophilized using xylol and molded in paraffin. The resulting molds were cut using a microtome into 3–5 μm clips which were then contrasted using the standard hematoxylin-eosin (HE) method.

Immunohistochemical methods

The resulting paraffin sections (3–4 μm thick) were dried at 56 ℃ for 16 hrs and deparaffinized in xylol, 100% ethanol, 96% ethanol, and distilled water, successively. Antigen unmasking was performed by transferring the deparaffinized samples into a plastic cuvette with 250 mL of citric buffer solution (10 mmol/L; pH 6.0), then cooked in a microwave oven two times successively for 5 min at maximum temperature. It was then cooled for 30 min at room temperature in a citric buffer. Endogenous peroxidase activity was blocked by submerging the samples in 3% hydrogen peroxide solution dissolved in distilled water for 5 min, after which they rinsed with distilled water and covered with phosphate buffer (0.02 mol/L; pH 7.0) successively three times for 2 min. The immunohistochemical procedure was done following the manufacturer's instructions using a commercially available kit (labeled streptavidin-biotin (LSAB) method, DAKO, Denmark). The samples were contrasted using the Mayer hematoxylin. Four antibodies (CD3, CD20, CD68, and C4d) were used in a 1:75 ratio, for which there is an external and internal positive control. Specific binding of

antibodies to certain antigens manifests in brown color, while hematoxylin nonspecifically binds nuclei and other cellular structures, coloring them blue. HE and immunohistochemical colored sample analysis was done using an optical microscope (Bx50F4, Olympus Optical, Japan).

Statistical analysis

Statistical data processing was done using the IBM SPSS Statistics computer program (SPSSInc., Chicago, IL, USA). The degree of correlation between clinical and morphological parameters and the TRS was calculated using the Spearman correlation test. Differences between variables with a significance value of ≤ 0.05 were deemed statistically significant.

Results

The following results were obtained by analyzing the data on the examined patients: the average age of the examinees was 45.94 years; the average age during transplantation was 42.72 years; 90.6% of patients were male and 9.4% female.

The prevalence of the most commonly diagnosed heart diseases among the respondents is shown in Table 1.

Table 1

Prevalence of most commonly diagnosed heart diseases among respondents

Diagnosis	% of respondents
Dilated cardiomyopathy	59.4
Viral myocarditis	21.9
Ischemic heart disease	12.5
Non-compaction cardiomyopathy	3.1

INTERMACS profiles are shown in Table 2.

Table 2

Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profiles among the examined patients

INTERMACS profile	% of respondents
1	4.2
2	20.8
3	8.3
4	54.2
5	12.5

NYHA scores are shown in Table 3.

Table 3

New York Heart Association (NYHA) scores among the examined patients

NYHA score	% of respondents
1	3.1
2	0
3	21.9
4	75

The average values of the EF after three, six, and twelve months after transplantation are shown in Table 4.

The Spearman's correlation test showed a negative correlation that was not statistically significant (r = -0.065; $p > 0.05$) between the TRS value and the EF difference value three, six, and twelve months after transplantation.

Table 4

Middle value of ejection fraction (EF) before heart transplantation, and after three, six, and twelve months after heart transplantation

EF	Middle value of EF (%)
Before transplantation	18.81
After transplantation (months)	
3	68.68
6	68.64
12	67.96

The average TRS was 0.42. The TRS was calculated as the quotient of the total number of rejections and the number of biopsies during a year. The histopathological presentations of grades 1R and 2R are shown in Figure 1 and Figures 2 and 3, respectively.

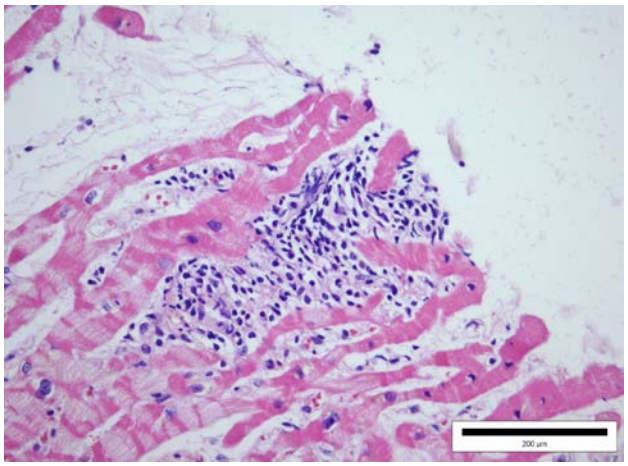

Fig. 1 – Grade 1R: perivascular lymphocyte infiltrate with a single focus of cardiomyocyte damage (hematoxylin and eosin staining, magnification ×40).

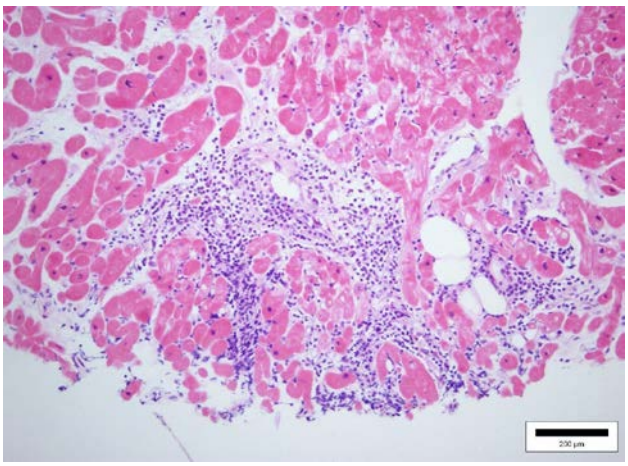

Fig. 2 – Grade 2R: the focus of interstitial infiltration with a large number of lymphocytes with a focus on cardiomyocyte damage (hematoxylin and eosin staining, magnification ×20).

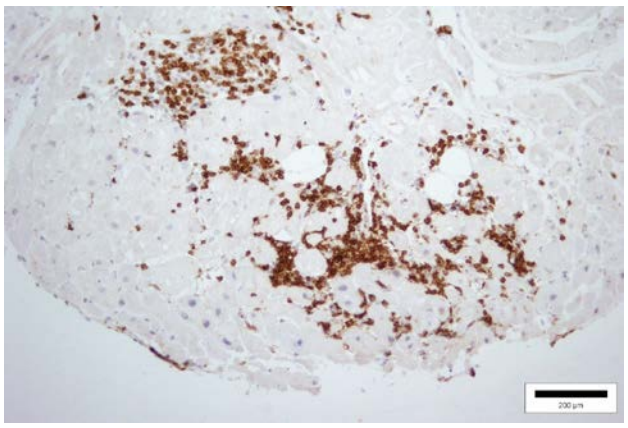

Fig. 3 – Grade 2R: the focus of interstitial infiltration with a large number of lymphocytes with a focus on cardiomyocyte damage (immunohistochemical staining for CD3, magnification ×20).

Immunohistochemical analysis showed the presence of C4d deposits in more than 50% of analyzed capillaries (Figure 4).

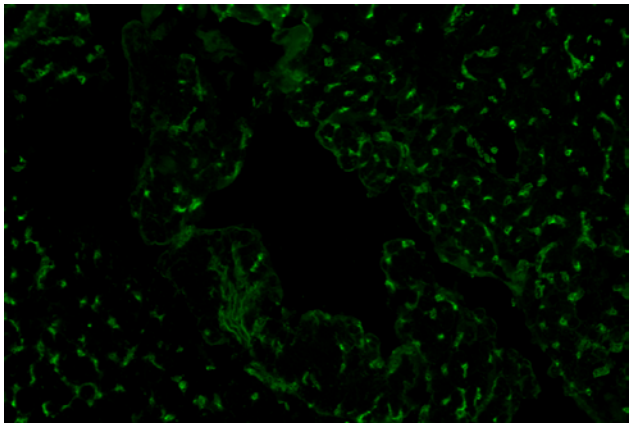

Fig. 4 – Antibody-mediated rejection: C4d deposits in more than 50% of capillaries in the endometrial biopsy sample (immunofluorescence microscopic analysis, magnification ×20).

AMR was observed in only one subject (Figure 5).

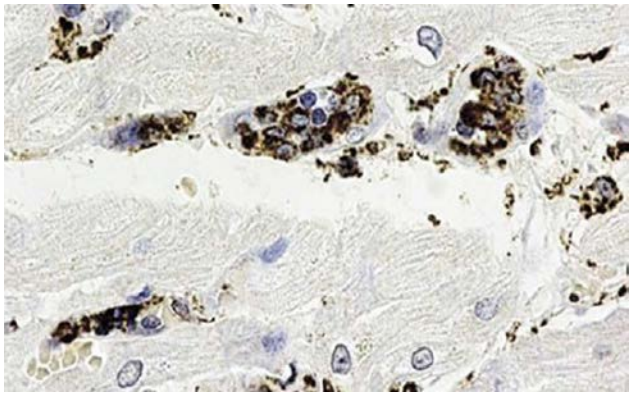

Fig. 5 – Antibody-mediated rejection: the presence of intravascular CD68-positive macrophages in more than 10% analyzed capillaries (immunohistochemical staining for CD68, magnification ×60).

Discussion

Despite the success of heart transplantation as a therapy for end-stage heart failure, acute rejection continues to reduce long-term survival in transplant recipients. Acute rejection develops as a consequence of recognizing histocompatibility antigens and the immune response to allogeneic heart muscle leading to progressive dysfunction and graft loss. EMB is a part of the current standard for monitoring complications and graft rejection and is based on the guidelines of the ISHLT. The highest incidence of HTR occurs in the first year after transplantation. Patients experiencing HTR grades of 2R and 3R in this time frame show poorer five-year survival. Several studies have drawn attention to the discrepancy between histological grade and graft function, especially in settings where declining cardiac function and increased mortality persist independently of grade [9–11].

The age distribution of the respondents in our study correlates with the literature data, which shows that the mean age of the patients is 50.5 years. Literature data show that 68% of patients with heart transplants are male. Dilated cardiomyopathy and ischemic heart damage are the most common causes that lead to the need for transplantation worldwide, which corresponds to the results of our research [12].

Clinical criteria that define eligibility for heart transplantation are partly the subjective discomfort of patients and partly defined on the basis of hemodynamic parameters at rest, and correlated with the NYHA classification. The NYHA classification, as a measure of functional capacity, is a subjective and often non-reproducible index, which varies day to day depending on various factors [13]. Our research showed that the largest number of subjects has an NYHA score of 4. NYHA score 4 and NYHA classification do not determine the best therapeutic approach (medical and pacing therapies, mechanical circulatory support, or heart transplantation); therefore, INTERMACS classification is used. Based on the competence of the prescribed medical therapy, hemodynamic and laboratory parameters, patient outcomes, and risk-benefit ratio, seven profiles have been defined for the INTERMACS. In our study, the majority (54.2%) of patients belong to profile 4 according to the INTERMACS classification [14, 15].

In our population of heart transplant patients and in accordance with the current immunosuppressive therapy regimen, allograph rejection is experienced by about 50% of patients at least once during the first year after transplantation. Monitoring a patient after a heart transplant is essential for the long-term survival of these patients. The gold standard for diagnosing rejection is EMB.

However, EMB is an expensive and invasive procedure that is partly limited by sampling error, as well as the existence of pathologist variability in assessing the degree of rejection. With significant advances in biotechnology, we are now able to explore the relationship between recipient and allography at multiple levels (genomic, epigenetic, transcriptional, proteomic, metabolic, immunophenotypic).

With all this in mind, we can expect that, in the coming period, a detailed description and determination of the meaning of genetic variants will lead to the development of numerous biomarkers (but also multimedia tests, such as genetic and epigenetic analysis) that can assess the patient's risk of continued transplant rejection. All of the above, with carefully balanced immunosuppressive therapy and adequate patient monitoring, can contribute to the better survival of heart transplant patients.

Conclusion

The average TRS obtained in this study is 0.42 and indicates a dominant cell-mediated graft rejection.

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