The Evaluation of the Intramyocardial Coronary Microvasculature Subsequent to Heart Transplantation Using Microscopic and Immunohistochemical Methods

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Introduction: This paper presents some histologic and immunohistochemical aspects of the intramyocardial microvascular network performed subsequently to cardiac transplantation in Romanian patients.

Material and method: We investigated a group of 30 heart transplant patients, aged between 12 and 58 at the time of transplantation, with an average age of 39 years, 23 male and 7 female patients. We studied in these patients the affection of the intramyocardial microvascular network subsequently to cardiac transplantation, using histologic and immunohistochemical standard methods. We followed the occurrence of foam cell arteritis at the level of large epicardial coronary arteries, as a direct sign of chronic rejection, lesions detected post-mortem at necropsy.

Results: In our study the most frequently detected microvascular lesions were the endothelial cell alterations, vascular wall thickening and severe perivascular fibrosis. Ischemia and reperfusion lesions with slight microvascular impairment were present in all patients in the first post-transplant biopsies (first 3–6 weeks). Acute cellular rejection developed in 6 patients in the middle and late period, in these cases we detected vasculitis lesions at the level of endomyocardial biopsies. Chronic rejection has affected three patients, who presented lesions at the level of large epicardial coronary arteries, as well as at the level of the small intramyocardial ones, such as wall thickening or perivascular fibrosis.

Conclusion: Subsequent to heart transplantation, small intramyocardial vessels are affected by immunological (rejection) or non-immunological factors (ischemia and fibrosis). Histologic and immunohistochemical study methods applied at the level of endomyocardial biopsies or post-mortem are useful for accurate assessment of small intramyocardial vessels and their involvement in the viability of the allograft.

Keywords: heart transplantation, intramyocardial microvascular network, immunohistochemistry

Introduction

The intramyocardial microvascular network is the final stage of metabolic exchanges between myocardiocytes and blood flow. The interstitial localization of small vessels assures a uniform distribution of the blood column around each myocardial cell. Lesions of the small vessels influence the normal functioning of the contractile myocardium as well as the cardiac conduction system [1].

Coronary microvascular disease (MVD) affects the smallest intramyocardial vessel and it is a different entity from the classical concept of coronary heart disease (CHD).

Recently published studies demonstrated that post-transplantation affection of the microvascular network is the result of the conflict between the immunologic and non-immunologic risk factors, ischemia and rejection being the most frequently implicated factors. Histological aspects of the intramyocardial small vessels (arterioles and capillaries) at the level of endomyocardial biopsies are correlated post-transplant with pathophysiological changes, which are detected by laboratory investigations (ECG, intracoronary Doppler flow velocity and aortic pressure) [2,3].

This paper presents a histological and immunohistochemical study of the intramyocardial microvascular network performed after heart transplantation in a group of patients from Romania.

We analyzed the degree of small vessel damage: arteriolar obliteration and capillary density in relation to histopathological lesions diagnosed post-transplantation. In addition to these, we assessed whether the use of immunohistochemical methods for evaluating small blood vessels at the level of endomyocardial biopsies was appropriate in all cases of ischemia or rejection.

Material and method

In our study we examined a group of 30 heart transplantation patients from the Institute of Cardiovascular Diseases and Transplantation of Tîrgu Mureş, whose histopathological monitoring was performed by the Pathology Department of the Clinical Emergency County Hospital, Tîrgu Mureş, between 1999 and 2010.

Tissue fragments were processed by standard histological methods: formalin fixation, inclusion in paraffin, sectioning, Hematoxylin-Eosin (HE) and special staining – Van Gieson's, Masson's trichrome and Orcein staining method.

We added to these immunohistochemical methods in order to emphasize the vascular endothelium in normal conditions, in conditions of ischemia, reperfusion or in case of rejection: CD31, CD34 and VEGF (Vascular Endothelial Growth Factor). As a staining protocol we used the ABC method (avidin-biotin complexes or streptavidin

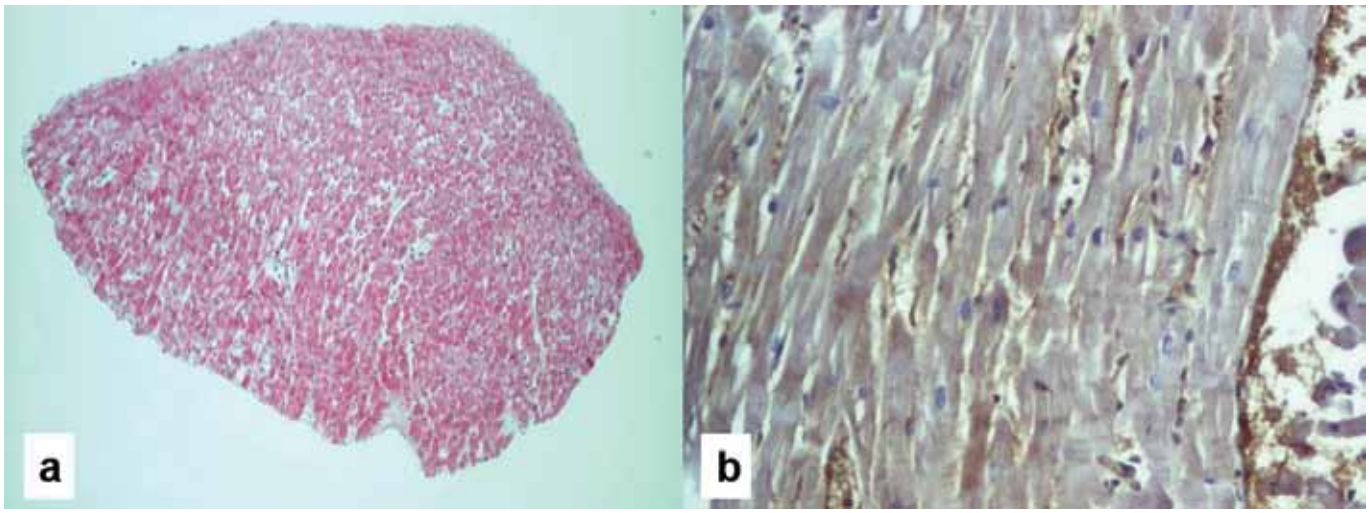

Fig. 1. Ischemia and reperfusion lesions, HE stain, Ob 10x (a) and intramyocardial microvascular network, Immunohistochemistry, VEGF (b)

biotin), chromogen visualization and nucleus overstaining with Hematoxylin.

The investigated histological lesions in our study comprise the aspect of endothelial cells, functional state of the vascular wall, interstitial modifications. As a direct sign of chronic rejection we monitored the occurrence of foam arteritis at the level of large subepicardial vessels, lesions detected post-mortem at necropsy.

Results

All patients underwent clinical, paraclinical and histopathological monitoring for the entire period of survival. Each patient went through 3–20 endomyocardial biopsies, according to the survival period and complications that occurred. In case of death, clinical anatomical necropsy was performed.

General description of the study group: 30 patients, aged between 12 and 58 years at transplantation, with an average age of 39 years, 23 male and 7 female patients. The indication for cardiac transplantation was in most cases dilated cardiomyopathy (26 cases), followed by ischemic cardiomyopathy (4 cases). Four patients died during the early and immediate period of treatment (first day – first month). These patients were not included in the study of small vessel lesions. Three other patients died during the late period (after the first year of treatment), with a survival period ranging from 2 to 3 years. Clinical anatomical necropsy was performed in these patients. After the first year of treatment, the survival rate in our group reached more than 70%, the highest survival period being so far more than 11 years.

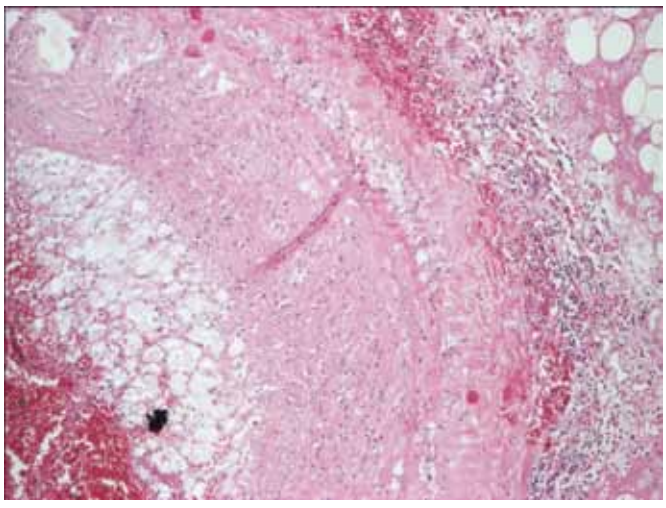

Fig. 2. Association between sever acute cellular rejection and chronic rejection HE stain, Ob 10x

Ischemia and reperfusion lesions were present in all patients comprised in our study, at the level of first 1–2 biopsies performed post-transplantation during the first 3–6 weeks after the surgical intervention.

Intramyocardial small vessels — arterioles, capillaries and venules — were described and counted separately for each biopsy. Their aspect was quasi normal or we found ballooning of the endothelium, thickening of the wall, stasis or even microthrombosis.

Immunohistochemical reactions were applied to 60 endomyocardial biopsies (the first 1–3 biopsies of each patient, except for those who died during the early or immediate period). Immunophenotypic analysis revealed that most of the endothelial cells presented immunoreactivity for VEGF and focal immunoreactivity for CD31 and CD34.

There were 6 patients in our group who underwent one to five episodes of acute cellular rejection during the interim (first month – first year) and late (after the first year) period. The rejection grade was mild (1R) or moderate (2R) according to ISHLT (The International Society for Heart and Lung Transplantation) 2005. Three out of the 6 patients died afterwards due to severe acute rejection (grade 3R). The first patient died because of neglecting to follow immunosuppressive treatment, the second because of severe acute cellular rejection, which did not respond to immunosuppressive treatment and the third one because of the association of sever acute rejection(grade 3R) with chronic rejection – cardiac allograft vasculopathy (CAV).

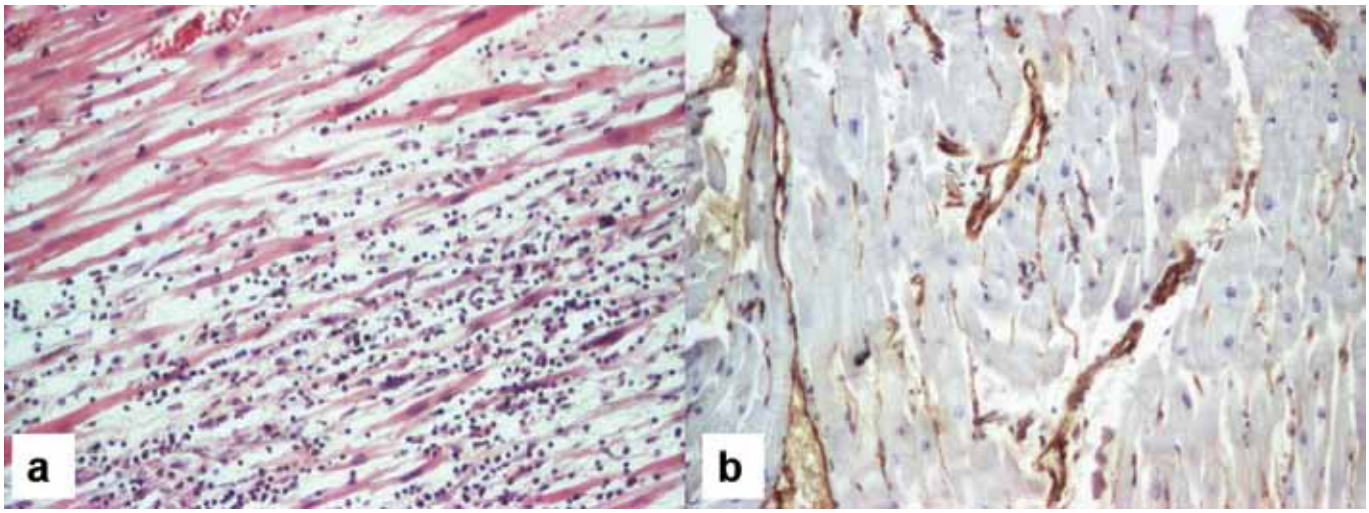

Fig. 3. Acute cellular rejection grade 3R - inflammatory infiltrate composed of T lymphocytes, HE stain, Ob 10x (a) and intramyocardial microvascular network, Immunohistochemistry, CD31 (b)

In these cases the intramyocardial vessels presented specific lesions: inflammatory infiltrate composed of T lymphocytes located perivascular or infiltrated through the whole thickness of the vascular wall, with or without endothelial damage — characteristic of vasculitis. Immunohistochemical reactions were applied to 22 cases of endomyocardial biopsies (in the event of acute cellular rejection) and to myocardial fragments collected postmortem (three cases). Immunophenotypic analysis revealed that most of the endothelial cells presented immunoreactivity for VEGF, CD31 and CD34, associated or not with the phenomena of vasculitis.

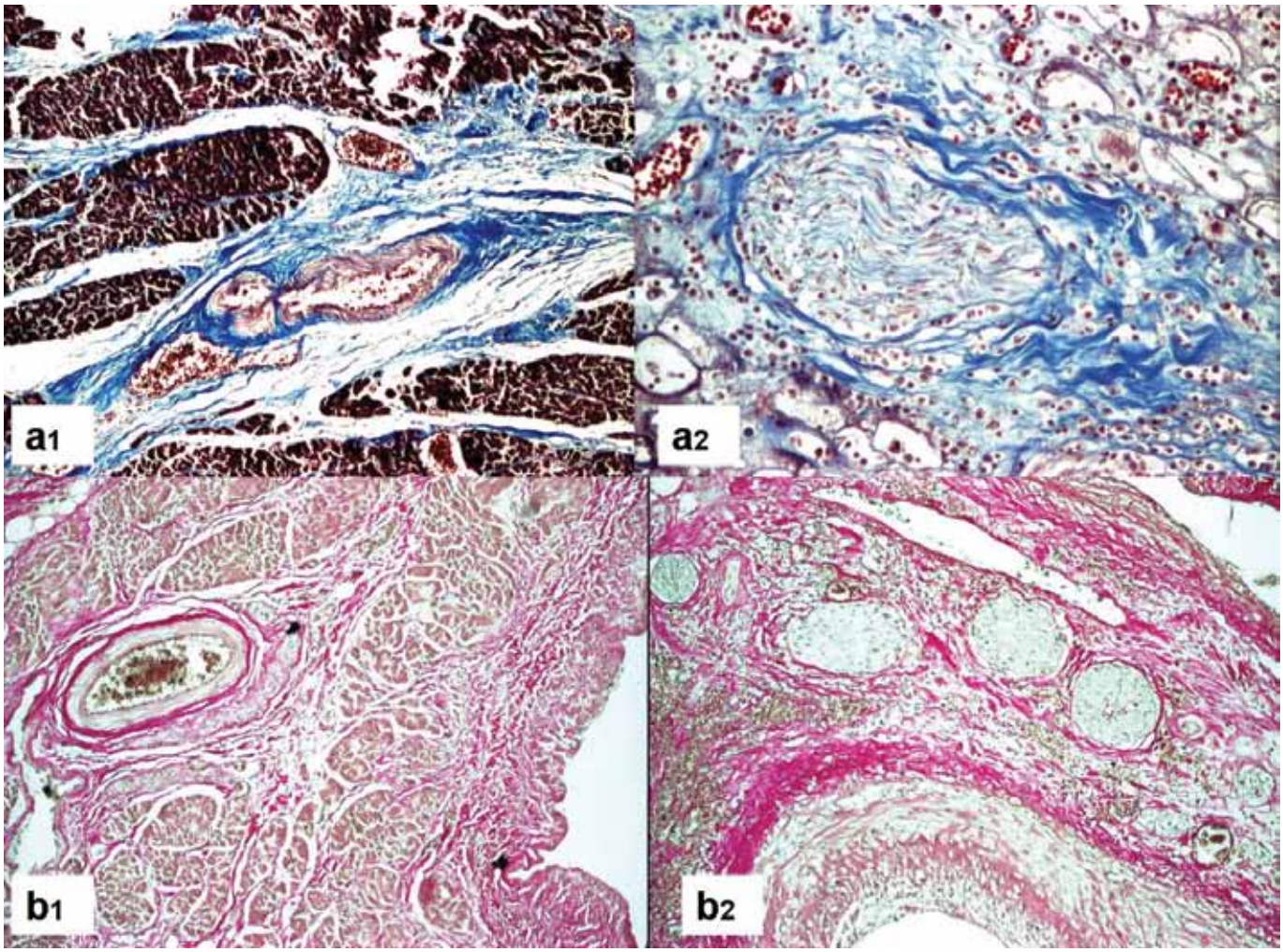

Fig. 4. Intramyocardial microvascular network - perivascular, perymiocytic and perineural interstitial fibrosis, Van Gieson's (a) and Masson's Trichrome (b) staining method, Ob. 4x

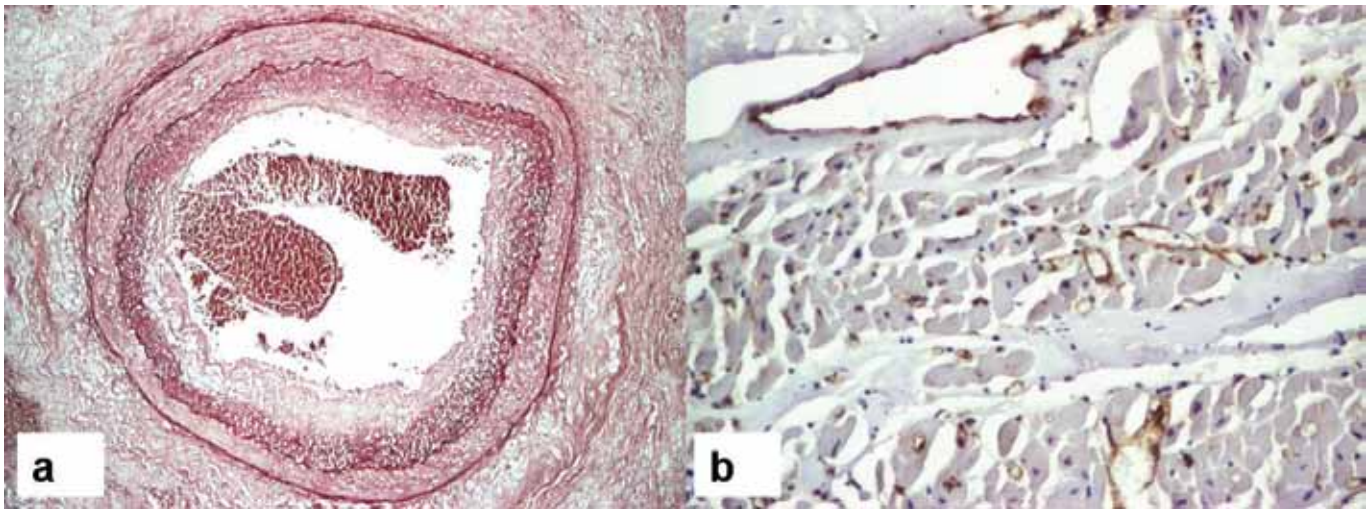

Fig. 5. Chronic rejection - CAV – subepicardial arteries, HE stain, Ob 4x (a) and intramyocardial microvascular network, Immunohistochemistry, CD 31 (b).

Chronic rejection, also called cardiac allograft vasculopathy (CAV), usually occurs during the late period (after the first year). In our study this occurred in three patients, followed by death and in one case associated with severe acute cellular rejection (grade 3R).

In these cases we found the affection of the microvascular system, chronic ischemic events at the level of myocardiocytes and interstitial fibrosis. When associated with acute cellular rejection vasculitis lesions also occurred. Orcein stain at the level of the subepicardic large coronary arteries allowed the differential diagnosis of native atherosclerosis.

It is to be remarked the subendothelial aspect with rich inflammatory infiltrate, made up of T lymphocytes and macrophages, located between the intima and media at the level of large subepicardial blood vessels. Trichromic stains — Van Gieson's and Masson's Trichrome — clearly evidenced interstitial fibrosis, which can present perymiocytic, perivascular or perineural predisposition. Fibrosis is often associated with chronic ischemic events and microvascular affection, which is considered to be an indirect sign of the development of chronic rejection.

Table I. The results of the study, synthetised

No. of cases (n=30), gender: 23 M, 7 F, age: 12–58y, mean age 39 y				
Histopathology	Immunohistochemistry	CD 31	CD 34	VEGF
Lesions of ischemia and reperfusion (n=30)				
Ballooned endothelial cells	Biopsy (n=60)	+	+	+++
Wall thickening				
Microthrombosis				
Acute cellular rejection (n=6)				
Wall thickening	Biopsy (n=22)	++	++	++
Vasculitis	Autopsy (n=3)			
Perivascular lymphocytic infiltrate				
Chronic rejection (n=3)				
Wall thickening	Biopsy (n=10)	+++	+++	+++
Perivascular fibrosis				
Myocytic ischemic lesions				
Foam cell arteritis in the subepicardial region	Autopsy (n=3)			

Immunohistochemical reactions were applied to 10 endomyocardial biopsies (in the event of chronic rejection in the late period) and to myocardial fragments collected postmortem (three cases). Immunophenotypic analysis revealed that endothelial cells present immunoreactivity for all three antibodies used in the study (VEGF, CD31 and CD34) associated with ischemic-type myocytic lesions and positively correlated with foam cell arteritis diagnosed postmortem at the level of large subepicardial vessels.

In our study the most frequently detected microvascular lesions were the endothelial cell alterations, vascular wall thickening and severe perivascular fibrosis. Ischemia and reperfusion lesions with slight microvascular impairment were present in all patients in the first post-transplant biopsies (first 3–6 weeks). Acute cellular rejection developed in 6 patients in the middle and late period, in these cases being detected lesions as vasculitis at the level of endomyocardial biopsies. Chronic rejection has affected three patients, who presented lesions at the level of large epicardial coronary arteries as well as at the level of the small intramyocardial ones, such as wall thickening or perivascular fibrosis.

Discussions

There are a series of local or systemic factors that can produce the dysfunction of the endothelial cells and implicitly of the intramyocardial microvasculature [4–7].

Intramyocardial small vessels after heart transplantation are initially affected due to ischemia and reperfusion lesions, which are detected in the biopsies of almost all patients 1–2 months post-transplantation. During our study we identified in the first 3–6 weeks post-transplantation ballooning of the endothelial cells or focal microinfarctions, with or without microvascular affection.

In order to quantify the endothelial lesions of small vessels we studied the VEGF expression at the level of myocardial fragments sampled as endomyocardial biopsies or post-mortem during various postoperative periods. In patients whose ischemic lesions and reperfusion was more prominent on usual stain (HE), VEGF expression was more intense. VEGF was expressed in the endothelial cells as well as in the myocardiocytes or some inflammatory cells (T lymphocytes and macrophages) in case of rejection. At the level of biopsies in the late period at the same time with the occurrence of interstitial fibrosis the VEGF expression increased at the endothelial level probably as an indirect sign of chronic ischemia. CD31 and CD34 were intensely expressed by all endothelial cells of the subepicardial large and small vessels — arterioles, capillaries and venules. We detected increased VEGF expression on the first biopsies with moderate CD31 and CD34 expression, but subsequently in the same patients we detected an increased expression of the endothelial markers.

Acute cellular rejection graded according to ISHLT 2005 as mild (1R), moderate (2R) and severe (3R) influences decisively the microvascular network: endothelial modifications, vasculitis, microthrombosis or hyaline microthrombi [8].

In our study, we identified in case of acute rejection these types of lesions at the level of endomyocardial biopsies or postmortem as being grade 2R or 3R.

Chronic rejection or CAV that can be diagnosed only post-mortem at the level of large subepicardial vessels displays a series of indirect signs of ischemia present at the level of biopsies [9,10].

During our study the most frequent sign of chronic ischemia was the presence of interstitial fibrosis with perivascular and perineural distribution, associated with vascular wall thickening and reduction in number of small vessels compared to previous biopsies.

The cornerstones of our study are the accurate detection of microvascular affection after heart transplantation, the involvement of these small vessels in the viability of the allograft and patients' survival rate. The involvement of immunohistochemical methods for histological post-transplant monitoring allowed a qualitatively correct quantification of rejections or intramyocardial vessels.

The small number of investigated cases can be primarily the weak point of our study. Even if heart transplantation has been successfully performed in Tîrgu Mureș since 1999, we still have a small number of patients. Although for the total number of 30 patients included in our study, for over 11 years, more than 250 endomyocardial biopsies were performed and analyzed and the necropsy of deceased patients was also performed the biggest problem for statistical analysis and pertinent conclusions represented the small number of patients. It is to be remarked that the heart transplantation activity has been carried out continuously for over 12 years and up until present over 45 heart transplantations have been performed, with excellent survival rates and patients' quality of life close to normal.

Conclusions

Our study demonstrates that following heart transplantation small intramyocardial vessels are affected by immunological (acute or chronic rejection) or non-immunological factors (ischemia and fibrosis). Histologic and immunohistochemical study methods applied at the level of endomyocardial biopsies or post-mortem are useful for accurate assessment of small intramyocardial vessels and their involvement in the viability of the allograft.

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