UNIVERSITY OF MEDICINE AND PHARMACY, TÂRGU-MUREŞ
SCHOOL OF DOCTORAL STUDIES

DOCTORAL THESIS

“CARDIAC ALLOGRAFT PATHOLOGY. COMPARATIVE HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY, LESION GRADATION AND ANATOMICAL AND CLINICAL CORRELATIONS”

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I. GENERAL PART

1. BRIEF HISTORY OF THE FIELD OF TRANSPLANTATION
   1.1. International experience
   1.2. Romanian Experience

2. INDICATIONS AND CONTRAINDICATIONS OF CARDIAC TRANSPLANTATION

3. BASIC NOTIONS OF IMMUNOLOGY TRANSPLANT

4. LESIONS CLASSIFICATION OF CARDIAC ALLOGRAFT REJECTIONS
   4.1. HANNOVER Classification
   4.2. STANFORD UNIVERSITY Classification
   4.3. TEXAS HEART INSTITUTE Classification
   4.4. ISHLT Classification from 1990
   4.5. New ISHLT classification 2005

5. PATHOHISTOLOGIC DIAGNOSTIC CRITERIA AND GRADING OF CARDIAC ALLOGRAFT REJECTION

6. PRINCIPLES OF THE POSTTRANSPLANT IMMUNOSUPPRESSIVE THERAPY

II. SPECIAL PART
1. AIM OF STUDY

1.1. Immunohistochemical and pathohistologic monitoring of cardiac posttransplant evolution of patients, grading of lesions and anatomical clinical correlations.

1.2. Main objectives:

1.2.1. Quantifying pathological changes at the level of endomyocardial biopsies in patients undergoing heart transplant.
1.2.2. Analysis in terms of survival and/or frequency of posttransplant complications occurred in correlation with the disclosed pathohistologic aspects.

1.3. Secondary Objectives:

1.3.1. Standardizing applied pathohistologic methods.
1.3.2. Introduction, optimization and standardization of immunohistochemical protocols for posttransplant cardiac monitoring.
1.3.3. Introduction and standardization of digital morphometric analysis in immunohistochemical transplantation.
1.3.4. The development of a new pathohistologic bulletin in order to report pathological changes in endomyocardial biopsies.
1.3.5. Sequential statistical analysis of posttransplant cardiac complications in predefined periods of time.

2. MATERIAL AND METHOD

2.1. Statistical Methods
2.2. Standard histological methods and special colorations
2.3. Immunohistochemical methods
2.4. Digital morphometric techniques
3. SUMMARY OF RESULTS, ANATOMICAL CLINICAL CORRELATION AND DISCUSSIONS

The originality of this thesis consists in the new concept of approaching a large number, 127 cardiac transplantation cases, with an extensive casuistry of 2 centers in 2 countries (Romania and Hungary), over a longer period of time - 9 years and 15 years. Totally, there were 850 endomyocardial biopsies performed in the 2 groups of patients and over 2000 microscopic sections studied.

4 different types of survey methods were used in order to achieve our proposed target and to achieve the planned primary and secondary objectives: statistical methods, standard histological methods, immunohistochemical methods and digital morphometric analysis.

The obtained data systematized in databases, were statistically interpreted with the aid of inferential statistics using the EpilInfo and GraphPad Prism software programs, a sequential analysis of complications was performed within predetermined periods of time and the survival curves of Kaplan-Meier. The attained results are presented in numerous graphs and tables. The statistical data were correlated with histopathological lesions disclosed at the level of endomyocardial biopsies.

Standard histological methods used for post-transplant monitoring (hematoxilin-eosin, van Gieson, Masson's trichrom, orcein, methyl green pironin) were performed with modern immunohistochemical methods as a national premiere in cardiac transplantation for the above mentioned 2 countries.

There were 8 monoclonal antibodies applied: CD20, CD45RO, CD4, CD8, CD68, CD31, VEGF and HIF. The attained results were correlated with standard methods - HE and trichromatic staining (van Gieson, and Masson’s trichrom, methyl green pironin). The use of these staining methods allowed to highlight the phenomena of acute cellular rejection, chronic rejection and the differential diagnosis of lesions that mime cellular rejection (Quilty effect, ischemia and reperfusion modifications, some infections or lymphoid infiltrates).

Acute and chronic cellular rejections were quantified with the aid of: CD20, CD45RO, CD4, CD8, CD68.

The following antibodies were particularly useful: CD31, VEGF and HIF to evaluate the myocardial response to ischemia (early and late), the lesions of
ischemia and reperfusion, the focal point of micro-infarcts or subendocardial ischemic lesions.

The obtained results have enabled us to elaborate this hypothesis and according to this to use this combination not only post-transplant, but also to assess the degree of ischemia in non-transplant patients.

Following correlations between the aspect of lesions by standard methods and immunohistochemical aspects, the value of using these modern methods and their importance for establishing a correct and complete diagnosis was demonstrated, resulting that these methods are qualitatively superior to those classical and this way they may influence the post-HTx prognosis and quality of life.

In order to eliminate the natural subjectivity of interpretation of the human eye, I applied again for the first time as a national premier for the 2 countries in the field of cardiac transplant, methods of digital morphometric analysis at the level of segments with immunohistochemistry for cardiac post-transplants. Thus the proportion of cells involved in the post-transplant immunity has been established correctly: the B lymphocytes / T lymphocytes (CD20/CD45RO), the lymphocytes helper / cytotoxic lymphocytes (CD4/CD8), macrophages (CD68) ratio and vascular affection in acute or chronic rejection.

The application of this method with CD31 was particularly useful for the real assessment of the actual number and appearance of blood vessels, large and medium coronary branches, small intramiocardic branches or capillaries at the interstitial level.

These results were also correlated with standard histological methods and statistical data demonstrating the superiority of immunohistochemical investigations versus classical methods and the outstanding importance of the use of immunohistochemistry together with the digital morphometric analysis.

The obtained results are even more important as, until now there has never been performed in Romania and in Hungary a complex analysis of clinical, paraclinic, histopathological, immunohistochemical and morphometric data and established detailed anatomical-clinical correlations which show the need of histopathological monitoring post-HTx and close interdisciplinary collaboration.

As a final result of the study a new model of histopathological report or bulletin was proposed for use at the Anatomy Pathological Service of the Emergency Clinical University Hospital, Mureș starting with 2009.
The proposed sample contains entries that allow the collection of patients’ clinical data essential for a correct histopathological diagnosis.

This model contains the latest requirements of international guidelines for the evaluation of acute cellular rejection or humoral and chronic rejection.

An absolute novelty is the introduction of regular immunohistochemical methods to all patients who are suspected of rejection or lesions that requires a differential diagnosis based on the usual staining. There are other lesions disclosed at the level of biopsies provided in the final report having an important role in the proper assessment of the patient’s condition.

The major desideratum of this complex study lies in the implementation of new directions in the diagnosis and management of post-transplant cardiac complications during the early, medium and late period.

These will lead to the establishment of a regional and national database, which could be used for all heart transplant centers existing in Romania (Bucharest, Targu Mures, Timisoara) or those who currently practice cardiac or vascular surgery and in the future will be able to get involved in heart transplantation (Cluj-Napoca, Craiova, Iasi).

At the same time the experience of this interdisciplinary study in Targu Mures, the 4 doctoral scholarships during the 4 years at the Clinic of Cardio-Vascular Surgery and Transplant of the Semmelweis University, Budapest and especially within the 2 scientific research projects: CNCSIS type TD (Young PhD students) no. 542, September 2007 - March 2009 and MD-type no. 20, May-June 2008, as project director, allows the study of post-transplant cardiac complications to be continued and to extend these studies to other cardiac diseases (cardiomyopathies, ischemic coronary disease or congenital cardiac malformations with genetic determinism).
4. CONCLUSIONS

4.1. The development of a multi-complex statistical, histopathological, immunohistochemical and digital morphometric study with numerous interdisciplinary components and anatomical-clinical correlations.

4.2. The two studied groups comprise a total of 127 patients in group 1 from Târgu Mureș and lot 2 from Budapest, with very good reports of survival rates reported in the early stage - first month (> 90%), medium stage - first year (> 75%) and late stage after the first year for the studied period (> 56%).

4.3. The highest survival rate recorded up to present was 9 years (group 1) and 16 years (group 2), although the reported survival rate in the specialty literature is over 20 years.

4.4. Extreme ages of patients included in this study were 12 years (group 1) and 61 years (group 2), in the specialty literature extreme ages were reported starting from newborns to 79 years old patients.

4.5. The achievement of all (primary and secondary) objectives and completion of the purpose of this study.

4.6. Introducing for the first time at a national scale in the field of heart transplant immunohistochemical methods with 8 monoclonal antibodies (CD20, CD45RO, CD4, CD8, CD31, CD68, VEGF and HIF) and digital morphometric methods, which have proven to be superior to the classical methods (hematoxilin-eosin and special stainings) in the final histopathological diagnosis, thus improving the indirect survival rate and patients’ quality of life.

4.7. The issuance of a new histopathological report in line with new international guidelines, with its implementation in the clinical practice starting from 2009.

4.8. Highlighting the importance of interdisciplinary collaboration and histopathological post-transplant cardiac monitoring, with significant statistical values of the predictability index $p$ in the binary analysis of the number of rejection phenomena and their occurrence or histopathologically diagnosed complications and patients’ death ($p <0.05$ $p = 0.03354$; $p = 0.0021$, $p = 0.0006$).

4.9. This study represents a starting point for 2 future projects. Within the framework of these projects, based upon the already used methods, we can study cardiomyopathies and congenital cardiac malformations with genetic determinism and the relationship between post-transplant ischemic heart diseases or coronary and microvascular damages.
5. ADDENDA

5.1. Work protocols optimized for I-VIII Immunohistochemistry.
5.2. EUROTRANSPLANT NECROHEART protocol
5.3. New histopathological report

6. LIST OF ABBREVIATIONS

7. SELECTED BIBLIOGRAPHY - 301 bibliographic titles.