“CAROL DAVILA” UNIVERSITY OF MEDICINE AND FARMACY, BUCHAREST
DOCTORAL SCHOOL
MEDICINE

Dynamic interactions between micro-environment and cardiomyocytes in the cardiac ischemic pathology

SUMMARY OF THE DOCTORAL THESIS

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List with abreviations and symbols

CD = cluster of differentiation

CD34 = marker for blood vessels

CD105 = marker of activated endothelial cells

CD117 = stem cells marker

Col I / IV= collagen I / IV

CS = stem cells

CSC = cardiac stem cells

ECM = extracellular matrix

EGFR = epidermal growth factor receptor

HE = Hematoxylin Eosin

HVS = left ventricular hypertrophy

ICAM-1/CD56 = intercellular adhesion molecule

IHC = immunohistochemistry

IL = interleukin

IMA= acute myocardial infarction

IMC =cicatricial myocardial infarction

KI67 = cell proliferation marker

MMP-9 = matrix metal proteinase 9

PDGFR = platelet-derived growth factor receptor

PMN = polymorphonuclear neutrophils

SMA = smooth muscle actin

TGF-β = transforming growth factor β

Tn-C = tenascin-C

TNF = tumour necrosis factor

VG = van Gieson

VEGF = vascular endothelial growth factor

VEGFR = vascular endothelial growth factor receptor

VIM = vimentin

VS = left ventricle

Introduction

Cardio-vascular diseases represent the main cause of morbidity and mortality, despite the continuous improvement of the diagnostic methods and therapy in last decade. Nowadays in Romania 2 out of 3 adults have an independent cardiac condition or in association with other systemic lesions.

From the physiopathologic point of view, myocardial ischemia occurs due to occlusion of a coronary artery secondary to the rupture of a atheromatous plaque. Ischemia leads to deep molecular and ionic alterations followed by a severe decrease of the systolic function. Prolonged myocardial ischemia is accompanied by massive cardiac necrosis, which extends from the endocardium to the epicardium.

The human heart has a limited regenerative capacity and the healing of the ischemic myocardium ends with a fibrous scar formation. During the evolution of the post-infarction phenomena, one can distinguish 3 stages: a marked inflammatory response (which cleans the necrotic debris from the infarcted area), a proliferative phase (characterised by diminished inflammation, myofibroblastic proliferation and occurrence of granulation tissue) and the fibrous scar formation.

The intercellular space, also known as extracellular matrix (ECM) is an unique dynamic and complex micro-environment, composed of a large variety of non-cardiomyocyte cells, such as: fibroblasts, myofibroblasts, pericytes, endothelial cells and stem cells, scattered in a heterogenous proteic network, which includes collagen fibers, fibronectin, proteoglycans, matrix metal proteinase (MMP-ase) and surface proteins for cellular adhesion. The most dynamic non-cardiomyocytic cell population is the fibroblast population, which are support cells in close contact with collagen fibers, that creates a 3-D mecano-sensitive network with support role for cardiomyocytes.

During the evolution of the myocardial infarction, the fibroblasts undergo a quick phenotype transition from a secretory phenotype to a myofibroblastic phenotype, that produce collagen and secrete paracrine factors, which are essential in scar formation and cardiac fibrosis. In the beginning, the post-infarction ischemic myocardium heals by collagen 3 deposition (reticulin fibers), which is brittle, then it is substituted in time with type 1 and 2 collagen, which is much sturdy, leading to substitution fibrosis.

Simultaneously occurs an angiogenic proliferation with high micro-vascular density, which can be demonstrated by means of IHC using EGFR, MMP-9, CD34 and TGF-β in the

residual myocardium, associated with an increased variability of IHC expression of certain adhesion molecules in the ECM, such as ICAM-1/CD56).

Ventricular remodelling that succeeds the myocardial infarction represents the structural basis of the ischemic cardiac failure and consists of certain changes regarding the size, shape, function, molecular and cellular composition of the left ventricle.

The main factors involved in cardiac remodelling are represented by the size of the infarcted area and the reparatory response post-MI. The confinement of the ischemic area is realized through early reperfusion. On the other hand, in order to manipulate the cardiac reparatory response (which involves complex mechanism of cellular and molecular interaction), it is necessary to have a deep knowledge of these complex interactions.

Cardiac regeneration is under the control of complex intra- and extra-cellular mechanisms, which can activate new synthesis pathways for cardiomyocytes, with new therapeutic perspectives in the future.

Cardiac ischemic lesions are associated with cardiac interstitium expansion, which can be afected both in cantitative composition (excessive proteic synthesis) and calitative changes, leading to alteration of the ratio between fibrilar and non-fibrilar collagen molecules, accompanied by marked changes of the biochemical composition of ECM.

The ECM components interact continuously in a dynamic way with cardiac cellular elements, providing a stable structural network, with a role in coordination of the intercellular humoral response.

Work hypothesis

Because the healing of the post-ischemic myocardium occurs due to a fibrous scar formation, the modern research focus on new possibilities of cardiac repair looking for re-establishing the heart electro-mechanic function. Among the innovative methods there are certain proposals: stimulation of endogenic cardio-myocytes by modulation of the signaling pathways and regulation of the cell-cycle elements, reprogramming of the stromal cells by expressing some cardiogenic factors, stimulation of the stem cells from the support stem cells niches or involvement of exogenous stem cells.

Until now, the field researches focused on the study and characterisation of cardiomyocytes, both on regarding the distribution at various cardiac levels and the functions achieved in relation with cardiac location.

It is important to identify and characterize the cardiac micro-environment and the non-cardiomyocytic populations due to their complex molecular interactions. The vast majority of the fundamental studies are performed on animal models and the results do not reflect the fiziopathology and morphology of the human cardiac tissue. Therefore, these results cannot be used in human therapy.

The present thesis tries to eliminate this inconvenient, using human tissues for study and correlating the clinical diagnosis with complex cellular changes. This thesis proposes a complex analysis of cellular and molecular cardiac components in ischemic myocardium on human tissues. The description and characterization of the cardiac acute ischemic lesions will allow in the future the treatment of patients with MI using early revascularization, associated with antifibrotic therapy in order to limit the necrotic area.

The study objectives

The general objectives of this study focused on morphologic analysis and immunohistochemical characterization of the cardiac microenvironment in ischemic lesions, centered on non-cardiomyocytic cell populations.

The achievement of the objectives was performed as follows:

- Documentation and establishment of the selection criteria in order to create the study batches
- Post-necroptic harvesting of the heart fragments
- Histologic assessement of the cases from the study batch
- IHC testing for VIM, SMA, CD34, CD56, CD68, CD117, MMP-9, Col I, Col IV şi Tn-C
- Identification and characterization of the fibroblasts from the left ventricle in control group
- Fibroblast and myofibroblast immunophenotyping in ischemic myocardium
- IHC identification of the inflammatory cells in ischemic myocardium
- IHC identification of the cardiac stem cells
- IHC characterization of the extracellular matrix
- Correlation of the achieved data and statistical analysis

The research was performed by means of 3 morphopathologic and IHC studies, that focused on histopathologic and immunohistochemical characterization of of the main components of the cardiac micro-environment in ischemic lesions.

The ECM plays an essential role in organising the tissue micro-environment, mediating cellular interactions, cell growth, cell migration and differentiation.

There are some limitations of the thesis, first of all of a material nature, because of lack of electronic microscopy and genetic tests for ultrastructual and bio-molecular analysis. All tissue fragments were obtained from autopsy, from patients with cardiac ischemia. In the future, the cardiac endobiopsy techniques will allow harvesting the myocardic tissue for IHC testing by means of an antibody panel, which can offer data about fibrotic process, inflammation, as well as cellular and molecular composition of the ECM.

General methodology of the research

This paperwork is a retrospective study which includes cardiac tissue fragments harvested form 78 dead patients during 2012 – 2021, with myocardial acute ischemic lesions, selected from 636 necropsies performed at the Department of Pathology from Emergency University Hospital, Bucharest.

The inclusion criteria were represented by: acute and chronic ischemic lesions, myocardial infarction in various stages of evolution and acute ischemic lesions associated with old myocardial infarctions.

The exclusion criteria were represented by: non-ischemic chronic cardiac lesions, such as sclero-lipomatosis, degenerative lesions, hypertensive cardiopathy, infections.

The control batch was composed of 10 cardiac tissues from 10 patients who died of non-ischemic cardiac lesions.

The informed consent was obtained from the patient relatives and the study was performed according to national legislation regarding the using of human cadavers for research purposes and the general principles of Universal Declaration of Human Rights.

From medical records of the patients, it was retrieved general data regarding the age, gender, clinical data. These data were correlated with the macroscopic and microscopic diagnosis, after the necropsy was carried out.

The paperwork is structured on 3 interconected histopathological and immunohistochemical studies, which aim at an exhaustive analysis of the cardiac micro-environment during ischemic lesions performed on a significant batch of tissue fragments.

The initial study aimed at the macroscopic and microscopic description, using standard HE and VG stains of the identified ischemic lesions, along with the analysis of the clinical data from the selected 78 patients for IHC characterization. The pre-existing cardiac

lesions are important because the patients with MI develop ventricular remodelling, characterized by ventricular dilation, associated with systolic dysfunction in a variable period of time. The dilation of the ventricular cavities occurs due to redistribution of the residual cardiomyocytes and changes of the interstital matrix under the influence of citokines released by the inflammatory cells.

In the study batch the age of the patients was between 47 and 95 y.o., with an average of 71.3, with a small male predominance (48 M: 30 F). The selected cases are represented in the chart form fig.1.

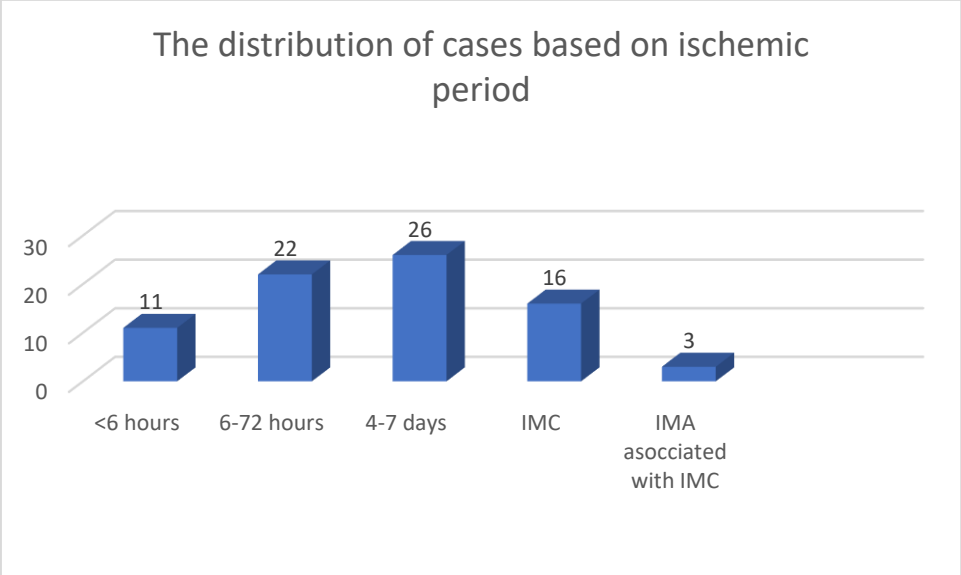

Fig.1: Case distribution in the study batch based on the period of the ischemic lesion

The studied cases presented ischemic lesions with variable location in the left ventricle, the majority being located in the antero-lateral and posterior wall (fig.2).

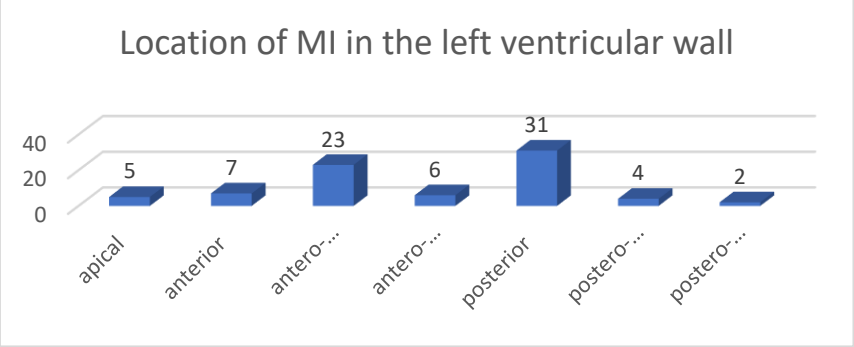

Fig. 2: Case distribution based on MI location

From the clinical data analysis correlated with the macroscopic aspect of the human heart during the necropsy, I ascertained that 92.3% of the patients (72 cases) presented signs of hypertrophic and dilatative cardiomyopathy.

In the analysed cases, the number of the patients with pre-existing lesions of dilatative cardiomyopathy was triple than the cases associated with hypertrophic cardiomyopathy. Aproximately ¾ of cases associated lesions of aortic and coronary lesions. Microscopically, cardiomyocytic necrosis is characterized by absence of the nuclei and intense cytoplasmic eosinophily, and disapearence of the cross striations.

Cellular destruction is followed by the infiltration of the necrotic area with numerous PMN, macrophages, lymphocytes and plasma cells, with the occurrence of granulation tissue (fig. 3).

In 2 cases, the ischemic lesions were associated with the rupture of the cardiac wall. The patients did not have comorbidities or pre-existing cardiac lesions before the ischemic episode. Microscopic, it was found a wide transmural myocardial infarction of 72 hours, a continuity solution in the epicardium with massive hemorrhage in the adjacent myocardium, which dissected and distorted the tissue architecture, accompanied by hypoxic lesions, vascular congestion, interstitial fibrosis and micro-hemorrhagic foci in the subepicardic connective tissue.

Classic optic microscopy revealed in all cases from the study batch, various degreees of interstitial fibrosis, from discrete collagen deposition to marked sclerosis, with distortion of the myocardic fibers. The fibrotic changes were demonstrated with VG stain in form of interstitial trabecule, with various shapes and sizes (fig.4).

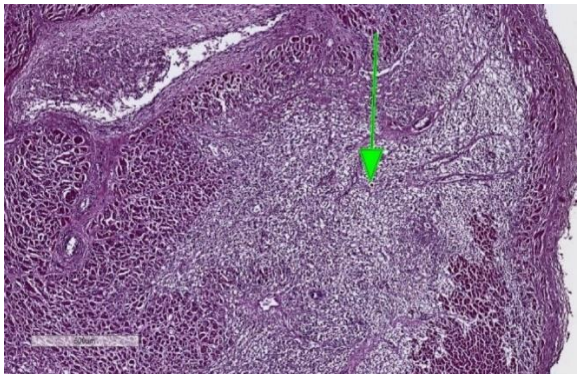

Fig.3. MI about 3-4 days; myocardic necrotic area replaced by granulation tissue and an adjacent vessel with thrombosis; HE, 40x

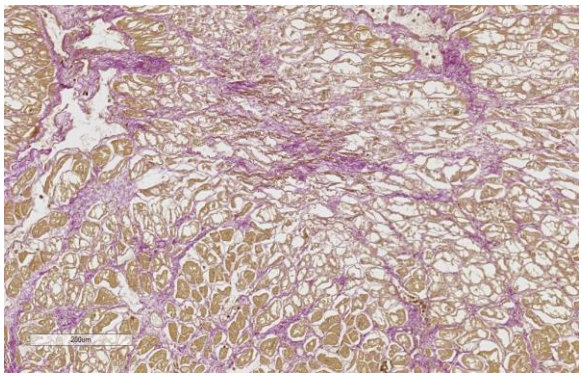

Fig.4. Hypertrophic cardiomyopathy; interstitial collagen bands with variable thickness; VG, 40x

The second study dealt with the analysis and characterization of the populations of fibroblasts, myofibroblasts and pericytes, stem cells and inflammatory cells form the cardiac interstitium involved in ischemic lesions, related to the evolution phases of the myocardial infarction. The IHC expression to vimentin, DMA, CD34 and cD68 was assessed in all cases with ischemic lesions from the study batch. These markers hav a role in the identification of the fibroblastic population, neonagiogenesis characterization and the inflammatory response.

In the first part of the study, I described the most dynamic and versatile cellular population, represented by the fibroblasts, which is in direct correlation with myofibroblastic population. Recent studies showed that the fibroblasts represent only 20% from non-cardiomyocytic cells, the most numerous population being represented by endothelial cells.

Due to the high resistance to ischemia, dynamic interaction with the cardiomyocytes and the high potential of mediators secretion, the fibroblasts are considered sentinel cells, which identify the ischemic lesions and lead to inflammatory reaction.

There are many populations of fibroblasts described in the literature: myofibroblasts, cytokine-secreting fibroblasts, phagocytic fibroblasts and fibroblasts with neoangiogenic properties.

I used IHC stain for vimentin, for fibroblast identification and SMA for myofibroblasts. Statistical analysis was performed. The most abundant population of fibroblasts was identified in patients with 3-4 days MI. In long term ischemia, the fibroblast population becomes depleted and is replaced by myofibroblasts. There were no variations related to the age of the patient.

In the cases with subendocardic MI, early reperfusion of the ventricular wall led to a rapid activation of the compensatory mechanisms. By means of IHC, I found that the number of fibroblasts grow rapidly and they are uniformly distributed into the peri-lesional myocardic interstitium.

On the other hand, in cases without early reperfusion, the total coronary obstruction led to an extended myocardial infarction, with marked transmural necrosis. In these cases, the fibroblast population was dramatically decreased, being destroyed by ischemia along with cardiomyocytes and other interstitial cells.

The myofibroblast population (staining positive to SMA) were in direct correlation with the fibroblasts and they were identified into the interstitium. These cells are medium-

sized, spindle-shaped cells, with centrally placed nuclei and reduced cytoplasm, with thin elongations (fig.5).

The inflammatory response is a complex process, which plays an essential role in cardiac healing. The initial phase lasts 3 – 72 hours after the ischemic event. This phase can be subdivided in 3 other successive stages: an “alarm” stage (characterized by releasing of proteic fragments from the necrotic cardiomyocytes), the leukocytic “mobilisation“ stage and the resolution stage.

The direct statistic correlation between CD68 and CD34 ($r = 0.534$, $p < 0.005$) could explain the role of histiocytic population in neoangiogenesis. The neonagiogenesis leads to accumulation of inflammatory cells in the injured area. The histiocytes stimulate the neoangiogenesis through PDGF synthesis, with role in increasing mitotic activity of the endothelial cells and muscular cells from the vessels wall.

In the study batch there was no correlation between the fibroblasts and macropahges, these 2 variables being independent. There was no significant statistical correlation between the histiocytes and myofibroblasts as well. Although both cell populations play an important role in cytokine synthesis, the population dynamics is independent.

Another cellular population, as well numerous and versatile as the fibroblast, analyzed in the second study was represented by the pericytes, which are IHC positive to SMA. These are vascular cells included in the microvascular basement membrane, which delineate the capillary walls, venules and arterioles. Besides the role in microvascular homeostasis, these cells are involved in a variety of cellular responses, such as: inflammation, fibrosis, tissue repair and tissue regeneration. The number of pericytes increases in MI, predominantly in the granulation tissue.

In the neoforming capillary walls from the granulation tissue was identified a numerous population of SMA positive pericytes. The ratio endothelial cells:perictytes = 3:1. The importance of identification and characterization of cardiac pericytes has a great significance for therapeutic potential in cardiac regeneration.

Because of their role in inflammation, neoangiogenesis and growth factors synthesis the pericytes can be used in the therapy of ischemic lesions. Experimental studies showed that intra-myocardic administration of pericytes could protect the heart from remodelling, due to their angiogenic and anti-apoptotic action, with paracrine effects on cardiomyocytes, fibroblasts and vascular cells.

Another line of research was the identification in the cardiac micro-environment of CD117 / c-kit positive endogenous stem cells, located in morpho-functional units, called CSC niches.

This morphologic entity (CSC niche) provides a regulatory structure for stem cells, and through the micro-environment, it stimulates cardiomyocyte proliferation, cellular migration and stem cells transformation in cardiomyocytes during the reparatory process.

Endogenous CSC represent a small population of immature cells, which have all charcteristics of stem cells: self-regeneration, clonality, multipotency, differentiation capacity into cardiomyocytes, smooth muscle cells and endothelial cells with specific genotypic features, located with predilection in the atrium and pericardium. They possess a specific IHC expression to CD117, CD34, CD105, Rex1, Nanog, Sox2 antibodies.

Comparative analysis of acute MI areas in contrast to chronic MI areas showed a slightly increasing of c-kit+ cells in the heart wth old ischemic lesions, which are clinically associated with various degrees of ventricular muscular hypertrophy (Fig.6).

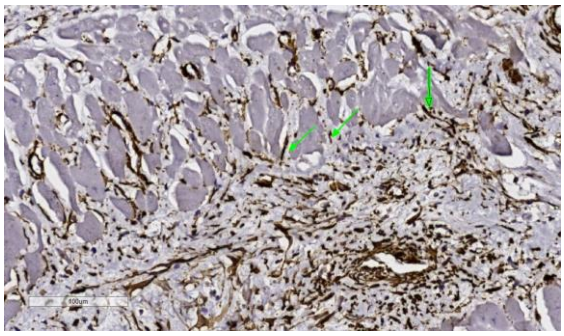

Fig.5. MI about 6 days; myofibroblasts proliferation; IHC, SMA, 200x

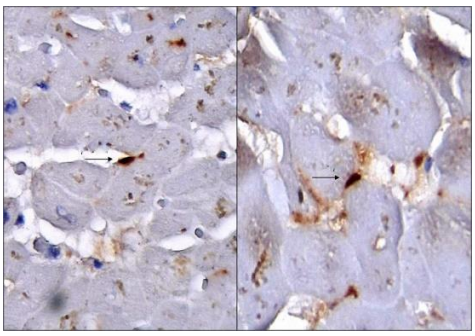

Fig.6. Old MI about 6-7 days; interstitial CSC; IHC, CD117, 400x

The third study focused on analysis and characterization of SCM composition, testing by means of IHC the main “actors” with remodelling role: Col I, Col IV, Tn-C, MMP 9 and CD34, on cardiac fragments in the study batch and the statistical correlation of the obtained data.

The normal collagen matrix is composed mainly of Col. I (70%), arranged in thick bands in the epimysium and perimysium, while Col III forms thin bands in the endomysium. The ECM composition has a determinant role in the evolution of regenerative processes. Cantitative and qualitative changes of the ECM leads to a high stifness of ECM, which implies the decreasing of left ventricular compliance, affecting the hemodynamics.

The myocardic fibrosis is the result of an imbalance in collagen metabolism, which leads to an increased collagen fibers synthesis, simultaneously with their gradually deterioration. In the fibrotic process are involved many cell types, either with direct action (myofibroblasts), or indirectly, such as macrophages, lymphocytes, endothelial cells, cardiomyocytes.

The research study targeted the dynamics of Col I, a fibrillar collagen, the main constituent of ECM, related to Col IV, a non-fibrilar collagen, secondary to ischemic events. In the residual myocardium I found abundant collagen deposition, initially type III brittle, collagen fibers, represented by reticulin fibers, which is then replaced with much sturdy non-fibrilar collagen such as collagen type I and II. The reticulin fibers form a loose continuous network, perpendicular to the cardiomyocytes, comparative to the collagen fibers, which are much dense, with a compact distribution, parallel to the myocardic fibers.

All the studied cases presented an increased amount of fibrosis in the ECM, compared to the control study. The cardiac fragments form the study batch presented a variable thickness of the subendocardic area, due to collagen deposition and myofibroblastic proliferation.

In a number of 45 cases, represented by 26 fragments with MI lesions about 4-7 days and 19 cases of MI about 7 days, with medium intensity of Col I deposition, I analyzed a comparative statistical analysis with SMA positive myofibroblastic population. In the analyzed cases, I ascertained a high Col I interstitial deposition, concomitant with the increasing of myofibroblastic population, possibly due to the role of this cell population in Col I synthesis and post-MI reparation (Fig. 7).

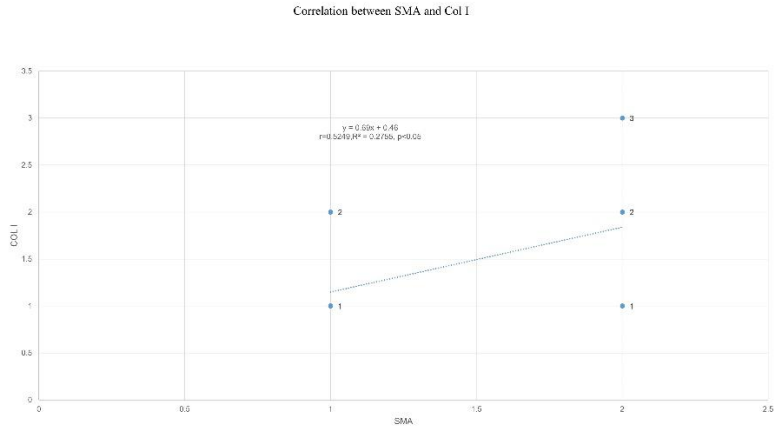

Fig. 7. Comparative analysis, statistically significant, between myofibroblastic cell population and Col I fibers

Comparative analysis between interstitial immune-expression of Col I and Col IV on the cardiac fragments with ischemic lesions, did not show statistically significant correlations.

This study demonstrated certain quantitative and qualitative changes in cardiac micro-vascularization. The robust angiogenic response after the MI begins in the myocardic tissue adjacent to the MI area and extends into the necrotic area. It was identified a thickness of the muscular layer of the large and medium caliber arterioles and interstitial collagen deposition around capillaries. Hypoxia secondary o ischemia leads to the increasing of micro vascular density in the context of granulation tissue. The neovascularization changes are characterized by occurrence of numerous small capillary vessels, with irregular lumen (fig.8).

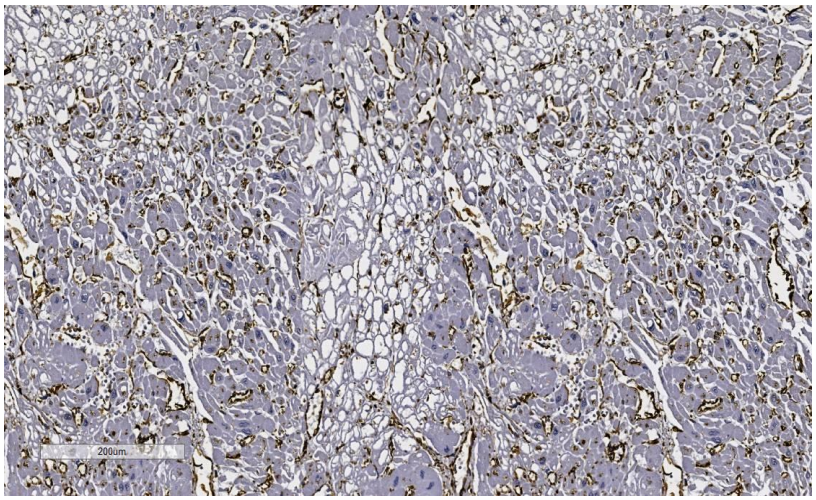

Fig.8. MI after 7 days of evolution: rich neovascularization network, with numerous small and medium vessels, developed around a chronic prolonged ischemic area; CD34, 100x

MMP are synthesized in the inflammatory phase of the ischemia, and they are involved in repairing and remodelling of the necrotic myocardium. This study also focused on MMP-9 expression in the ECM (MMP is a cytokine synthesized early in the ischemic myocardium).

In this study I identified by means of IHC, the early MMP-9 expression at tissue level in cases with MI less than 6 hours. In the study batch, the interstitial immune-expression of MMP-9 was high in ischemic lesions lup to 3 days. On myocardic fragments with more than 7 days ischemia, no MMP-9 expression was detected, which was in accordance with the literature.

Tn-C is a hexameric multi-modular protein, without IHC expression in normal heart, but secreted and deposited into the interstitium in remodelling processes. Tn-C immune-expression correlates directly with myofibroblastic presence around the area of infarct, supporting its role in ventricular remodelling after MI.

The direct statistical correlation between myofibroblastic population and Tn-C expression in RCM confirms the role of Tn-C in the inflammatory and proliferative phases of MI. Comparative analysis between interstitial immunoepression of Tn-C in MI with ischemic lesions up to 7 days and the macrophage population (CD68+) showed a direct correlation between these 2 markers.

Interstitial immune-expression of Tn-C in the peri-lesional residual myocardium correlated statistically with MMP-9 immunoexpression ($r = 0.53$). Taking into account that both MMP-9 and Tn-C secretion takes place early in the evolution of MI, both markers being well known for their predictive role in the occurrence of cardiac remodelling after myocardial infarction, I propose the IHC testing of these antibodies on myocardic byopsies in patients with acute ischemia, with the purpose of a targeted cardiac therapy.

Conclusions and personal contributions

In the present study the percentage of the necropsies of the deceased patients was 12.1% from the total performed autopsies among dead adult patients during 2012-2021, in the pathology department of the EUH (SUUB).

In the first study, I performed an exhaustive histological analysis on a batch of 78 cases with ischemic lesions. Three quarters of the analized cases associated important changes of coronary and aortic atherosclerosis.

The low regeneration capacity of the myocardium is compensated by a complex substitution fibrosis of the post-infarction cardiac healing involving dynamic interactions between cell populations and ECM.

In the second study, I performed the IHC identification and characterization of the interstitial non-cardio-myocytic cell populations. I found a direct corelation between fibroblastic and myofibroblastic cell populations, supporting the literature data.

The cardiac cell population with the most potential of phenotypic and therapeutic modulation is represented by fibroblasts. The activation of the fibroblasts represents a critical reparatory answer in keeping the cardiac geometry and function. The dynamic changes during the prolifrative phase generates biochemical signals which determines the conversion

of the fibroblasts into myfibroblasts, as well as the proliferation of a neoformation microvascular network.

In the cardiac interstitium I identified 3 main types of fibrosis: reparatory (substitution) fibrosis), reactive fibrosis and perivascular fibrosis. In the development of interstitial fibrosis there is a balance between fibrolytic factors (MMP and collagenase) and fibrogenic factors (collagen deposition). The substitution fibrosis implies the formation of a dense scar, resistant to the lytic factors, which increses the stifness of the ventricular walls, leading to arythmia and cardiac failure.

The complexity of the fibroblastic response associated with the heterogeneity of the fibrotic fiziopathologic conditions hinders the development of an anti-fibrotic strategy in ischemic cardiac lesions.

Analysing the relation between the histiocytic inflammatory infiltrate and formation of new vascular lumens, I found that there is a direct correlation between them, which explains the role of the histiocytic population in the formation of new vascular structures. The histiocytes stimulate neoangiogenesis through PDGF synthesis with role in increasing of the mitotic activity of the endothelial cells and muscular cells.

An important contribution is represented by the identification of the CSC in the cardiac interstitium, in the nearby of the cardiomyocytes and vascular capillaries, located in morpho-functional units called cardiac niches. The study regarding the analysis and characterization of the ECM has an innovative feature, due to the multitude of IHC markers and the focus on the interrelations among cellular elements of the ECM and humoral mediators during ischemia.

In the chapter 8 I performed the characterization of the post-ischemic interstitial fibrotic changes using IHC analysis of fibrillar collagen I synthesis and non-fibrillar collagen IV as well. Ischemic cardiac lesions and MI associate with dynamic changes in the structure and composition of the ECM. Using the IHC technique, I identified a series of cellular and structural elements represented by col IV & col I fibers, MMP-9, Tn-C and a high vascularization. These elements are involved in post-ischemic reparation, through fibrosis mechanisms, cardiomyocytic hypertrophy and post-IM remodelling. The complex interactions between these many factors generate a unique microenvironment which ensure the rhythmic cardiac function. Any inbalance in the matrix structure or composition affects the regulation of the cardiac network, followed by remodelling.

The synthesis of Col. I play role in making a sturdy network which maintains the cardiac integrity, limits the expansion of the necrosis and prevents remodelling.

The inhibition of MMP-9 secretion associates with decreasing of neoangiogenesis and fibrosis, being a valuable instrument in the anti-myocardial infarct therapy, to prevent remodelling and occurrence of cardiac failure.

The most important clinical aspect in patients with MI was represented by the post-necrotic ventricular remodelling, characterized by ventricular dilation associated with systolic dysfunction in a variable time period (weeks or months after the infarction).

The dilation of the ventricular cavities is explained by the rearrangement of the residual cardiomyocytes and the degradation of the interstitial matrix under the influence of MMP and inflammatory cells.

In the third study, I performed the identification of Tn-C in the cardiac interstitium during the evolutive phases of the ischemia, which were correlated with the clinical data of the patients.

Tn-C immunoexpression correlates directly with the myofibroblast population near the ischemic area, which shows the role of Tn-C in the direct recruitment of the fibroblasts in the ischemic area and post-MI ventricular remodelling.

Tn-C stimulates the fibroblast migration to the injured area, accelerates the transformation fibroblast / myofibroblast and promotes the reparatory phase of the myocardium. In the future, IHC tests of Tn-C on cardiac endobiopsy correlated with seric level of Tn-C could be used as predictive markers for cardiac remodelling in patients with cardiac ischemic diseases.

The cellular response in ischemic tissue was characterized by an expansion of ECM, a high matrix response and a high level of inflammation, correlated with a high chemotactic gradient for macrophage recruitment, that lead to an increased collagen synthesis and formation of a fibrous scar.

The thickening of the endocardium associated with the increasing of the subendocardic connective tissue represents an adaptative cardiac response to the volume changes of the left ventricle.

Identification of Col. IV in the necrotic area is an indicator for post-ischemic reparation process. Col IV distribution in the necrotic area is a dynamic process, depending on the period of ischemia.

Col IV distribution in the ECM was characterized by a randomly arrangement and a low stain intensity in pericardic area and in the residual peri-necrotic area, due to wide tissue destruction during prolonged ischemia.

There also were limitations of the study, such as: lack of electron microscopy and genetic investigations, the difficulty of harvesting the samples, which were taken exclusively post-mortem, without in vivo endobiopsy.

A future research direction could be the proteomic and functional characterization of the cell populations form the ECM of the human heart, “mapping” the cardiac cells, which is useful for targeted therapy.

Further studies and a detailed description of the epigenetic and angiogenic changes and apoptosis cascade of ECM in ischemic lesions and cardiac failure, along with regulatory role of the MMP, mRNA and TIMP will provide the key for a future targeted therapy in patients with ischemic lesions.

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