The Interplay between Cardiomyocytes and Non-cardiomyocytes in Postoperative Atrial Fibrillation

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Postoperative atrial fibrillation is a common complication after cardiac surgery, and changes in atrial structure may be the substrate for atrial fibrillation. In this study, including 10 coronary patients admitted at the Cardiovascular Disease Institute in 2012, we attempted to identify histopathological and immunohistological changes in right atrial tissue that might explain the development of postoperative atrial fibrillation. Atrial tissue samples from patients in the atrial fibrillation group were compared with samples from patients who remained in postopertive sinus rhythm. Lesions identification requires microscopic examination using special histological techniques and immunohistochemistry.Atrial tissue from 10 patients who underwent elective coronary artery bypass grafting was sampled. Histological and immunhistochemical methods were used for revealing atrial lesions. Five patients developed postoperative atrial fibrillation. Both groups of patients developed degenerative and adaptive lesions, but in different degrees, values recorded in postoperative atrial fibrillation being much higher than in postoperative sinus rhythm.Abnormalities in atrial biopsies can indicate the morphological substrate for postoperative atrial fibrillation development. The same lesions, with various degrees of intensity, make the difference between the severely ischemic myocardium in postoperative atrial fibrillation hearts compared to mild ischemia in postoperative sinus rhythm hearts.

Keywords: atrial fibrillation, immunohistochemistry, ischemic myocardium

Atrial fibrillation (AF) is a common complication after cardiac operations, occurring within the first postoperative week in patients undergoing coronary artery bypass grafting (CABG) [1].

The present study was designed to correlate any atrial lesion with the occurrence of postoperative atrial fibrillation (POAF), and thus to identify histological and immunohistochemical (IMH) markers in patients developing atrial fibrillation after CABG surgery.

Cardiomyocytes (CMs) and non-cardiomyocytic components, as a substrate of cardiac dysfunction and injuries, are insufficiently studied, especially the role of non-cardiomyocytic components in AF. These elements were investigated on animal experimental models and usually such results cannot be extrapolated to humans [2-4].

Identification of cardiomyocytes and extracellular matrix components is based on IMH techniques.

Alpha-smooth muscle actin (α-SMA) is temporarily positive, only early in the development of cardiac muscle fibers. In adults, α-SMA is expressed in subendocardial small smooth muscle fascicles and in excitoconductor structures [5].

Desmin is one of the markers early expressed during cardiac embryogenesis, the intensity of cardiomyocytes staining for desmin increasing progressively with age. Desmin is essential in maintaining the structure of cardiomyocytes [6]. A decrease in desmin-positive myocytes was reported in the myocardial tissue from patients with advanced ischemic disorders, compared with healthy people. The deficiency of this intermediary filament in the cytoskeleton was associated with a reduction in cardiac function and a poor prognosis and is used by some authors to point out acute ischemia zones on autopsy pieces [7].

CD34 is a cell-surface glycoprotein, which acts as an intercellular adhesion factor. It is a well-known marker of hematopoietic progenitor cells and endothelium, both in the fetal and adult periods [8].

CD68 is a useful marker for macrophage lineage. Macrophages are important in inflammatory processes in the coronary heart disease. Normal macrophage number varies significantly among patients (range 0±6 cells/high power field-HPF) [9]. The precise amount of macrophages within the diseased human heart is unknown.

Our study aimed to identify histological particularities and immunohistochemical expression of cardiac cells and non-cardiomyocytes under ischemic conditions in association or not with POAF.

Experimental part

Materials and methods

The study evaluated 10 patients hospitalized at the Cardiovascular Disease Institute, Iasi, for coronary surgery in 2012, including 7 men and 3 women. Patients' age ranged from 50 to70 years (mean age, 61.77 years). All patients gave their consent to participate in the study prior to cardiopulmonary bypass. Approval to conduct the study was obtained from the Institution's Board.

The ten coronary patients, 5 patients with POAF and 5 with postoperative sinus rhythm (POSR) were selected by clinical criteria: absence of transitory POAF, no concomitant hyperthyroidism, and no valvular diseases.

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Tissues samples harvested before cardiopulmonary bypass from the right atrial appendages (RAA) of the POAF patients were compared with samples from patients who remained in POSR. Pathology referring to atrial myocytes and connective tissue components was evaluated histologically and immunohistochemically.

Histological examination was done in accordance with existing standard protocols for paraffin-processed tissues by employing usual (hematoxylin and eosin-HE) or special (Sirius Red - SR) staining techniques. An Olympus CX41 light microscope (Olympus, Tokyo, Japan) was used for histological evaluation.

In cardiomyocytes, the histological examination included the assessment of CM size changes, due either to hypertrophy (HT) or myocytolysis, and the existence of other adaptive or degenerative cell lesions. In atrial interstitium, we assessed the degree of fibrosis, inflammation and capillary circulation, as well.

Immunohistochemistry was performed according to standard protocols for paraffin- embedded tissues. IMH examination focused on CM phenotype, inflammation and microcirculation, assessing alpha-smooth muscle actin (α-SMA), desmin, CD68 and CD34 markers.

Quantification of lesions was achieved by morphometric techniques, using a color image analysis system: Quick PHOTO MICRO 3.0. Collected data were processed by comparing histological and IMH variables from patients with POSR and POAF. The results were expressed as average values and percentages (small study groups).

Myocyte diameters. To evaluate CM size range, we assessed CM diameters on transversely sectioned muscle fibers displaying the nucleus in the section plane. An average myocyte diameter was obtained on 10 sections from each group, *measured* in all HPFs.

Myocytolysis, appearing as cytoplasmic vacuolation, was determined in CMs where vacuoles involved at least 25-30% of the cytosol. This lesion was estimated only in CMs whose nuclei were present in the cross section plane.

Fibrosis, which is the result of increased interstitial fibrous tissue, was quantified by relating the fibrous interstitial area (stained in red with SR dye) to the considered histological section area.

Microscopically, we analyzed 10 histological sections at high magnification (x400) for each case. Results were expressed as percentages or means of the number of damaged cells compared to the total number of nucleated cells. Fibrosis was determined by relating atrial fibrosis area to the entire histological section area studied on the HPF of view.

IMH analysis of the CM proteins, actin and desmin. Normally, α-SMA is a contractile protein of fetal phenotype, which is absent in the adult phenotype of CMs, while desmin is a characteristic protein of the adult phenotype of cardiomyocytes. Quantification was done by relating the total number of CMs with positive reaction to a specific protein to the total number of CMs in the histological sections we considered.

For assessment of inflammation, we used monoclonal anti-CD68 antibodies which are a CD68 pan-macrophage marker. Quantification was done by counting positive CD68 cells in the field investigated at 400x magnification (HPF).

Evaluation of the microcirculation. Immunostaining using monoclonal anti-CD34 antibodies specific to endothelial cells was performed to identify the capillaries. Microcirculation measurements were based on counting capillaries that reacted positively and turned brown in each HPF.

Immunohistochemically we analyzed 10 fields at 400x magnification for each case. The results were expressed as percentages or means of the number of positive cells as related to the total number of nucleated cells in the studied area.

Results and discussions

From 10 selected patients aged from 50 to 70 years, with chronic myocardial ischemia, 5 were with POSR and 5 with POAF. Average age of patients with POAF was higher (61.77 years) than that of patients who remained in SR after cardiac surgery (58.7 years).

The paper associated histopathological, immunohistochemical, morphometric and statistical studies.

On histopathological examination, the moderate increase in size of CMs by myocytolysis or HT was detected in most specimens: 4 out of 5 patients in the POAF group, and 2 out of 5 patients in the POSR group. High levels of lipofuscin were observed in myolytic CMs, in 4 out of 5 patients with POAF compared with 3 out of 5 patients who maintained SR (data not shown).

HT, meaning the increase in CM size, was present in both groups, but major differences were evidenced between them (26.23% in POSR versus 48.1% in POAF). Myocytolysis was estimated by assessing the extent of cytoplasmic vacuolation. We found different results in POSR (26.34%) and POAF (74.04%) (data not shown).

Several histopathological abnormalities were encountered within atrial interstitium, in both groups, the commonest being interstitial fibrosis (IF). IF, meaning increase in myocardial interstitium due to *excess fibrous* tissue growth, was detected in all 5 patients in the POAF group and only in 2 patients in the POSR group, respectively. Fibrosis was higher in POAF (20.11%) patients than in POSR (11.76%) ones. No interstitial inflammatory infiltrate was seen (data not shown).

Immunohistochemically, there is no marker with absolute specificity for either cardiomyocytes or non-cardiomyocyte cells.

In POSR myocardium with ischemic lesions, α-SMA was strongly expressed in all myocardial fragments at the level of intramyocardial coronary vascular walls, but also in the delicate bundles of smooth muscle cells particularly disposed in the subendocardium, or in excito-conductive structures. In CMs, we distinguished an increased α-SMA positive reaction at the periphery of myolytic CMs, reaching a higher intensity in the POSR group (14.02%) than in the POAF group (12.16%) (fig. 1 a, b).

Desmin was present in all the examined heart specimens, but cardiomyocyte staining was more extensive in POSR hearts in comparison with POAF atrial specimens. Positive reaction to desmin was somewhat lower in POAF (24.04%) than in POSR (27.21%), denoting greater loss of CM contractile function in AF (fig. 1 c, d).

Macrophages (Mfs) were identified within interstitial septa and perivascular areas. The number of macrophages varied slightly between the two groups of patients. No major inflammatory differences were observed between the two studied groups (6.5 in POSR; 7.87 in POAF) (fig. 2 a, b).

The vascular endothelium stained positive for CD34 in all cases (fig. 2 c, d), revealing a dense, capillary network. Positive reaction slightly decreased in hearts of patients with chronic ischemia and POAF (73.44/ HPF), compared to POSR hearts (75.16 /HPF).

POAF still remains one of the most common causes of morbidity after cardiac surgery [10]. Histological lesions were noted in most specimens, generally showing more advanced aspects in POAF patients than in POSR patients.

One of the most striking results of our study was the increased CM size, especially by vacuolation, in patients with POAF. CM vacuolation could occur in response to exposure of cardiac cells to hypoxic stimuli [11]. We

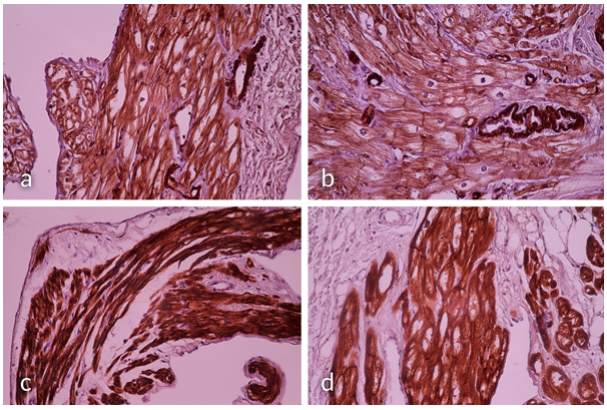

Fig. 1. (a) POSR, (b) POAF – atrial cardiomyocytes and vessels (α-SMA, x40); (c) POSR, (d) POAF - atrial cardiomyocytes (Desmin, x40)

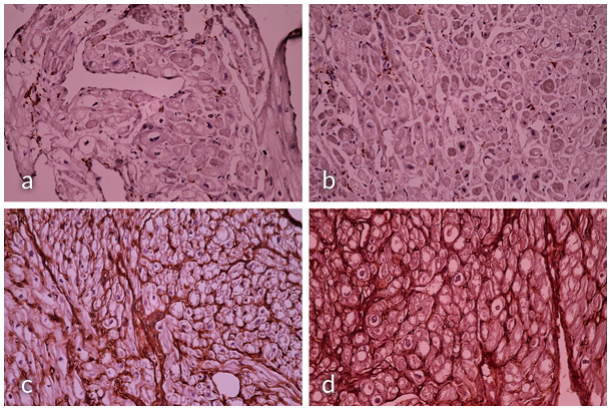

Fig. 2. (a) POSR, (b) POAF – interstitial Mfs (CD68, x40); (c) POSR, (d) POAF- interstitial capillaries (CD34, x40)

observed that both patient groups presented increased CM vacuolation as a possible arrhythmogenic substrate for development of POAF, but the feature was severe only in POAF specimens. The hypertrophy, as an adaptive reaction to hypoxia was twice as frequent in POAF patients as in the POSR ones.

In the POSR hearts, we found a lower expression of the α-SMA than in the POAF ones. Actin was expressed in the wall of intramyocardial coronary arteries. Concurrently, it was expressed in the delicate subendocardial smooth muscle bundles**,** as well as in Takeda' observation [12]. Desmin was expressed in all cases, the reaction being more or less intense at the periphery of atrial CMs, as some other authors also indicated [13]. So, desmin myocardial distribution was diffusely expressed in the CMs from POSR patients without cardiac symptoms and only focally in the POAF hearts with more severe ischemic lesions. On the contrary, α-SMA was more intensively and diffusely expressed in POAF and only focally identified in POSR specimens, similarly to CD34, as mentioned by other researchers, too [8].

Lubitz et al [14] showed that, irrespective of age, atrial fibrosis increases susceptibility to developing POAF. We consider fibrosis, representing a sign of ischemic myocardium, could decrease the tissue conduction.

Histologically, we didn't find any atrial interstitial inflammation, but changes in microcirculation and Mfs foci were present at the IMH–al analysis. Therefore we concluded that Mfs foci markers were associated with vascular injuries.

Considering the series focused upon, inflammatory reaction was reduced in POSR, but enhanced in POAF, reflecting ongoing inflammation and consequent fibrosis. This mild inflammatory process may be the tissue response to ischemic degenerative CM lesions, in the absence of a systemic inflammatory syndrome and a localized inflammatory infiltrate.

Lin et al [15] outlined that inflammation plays an important role in the pathogenesis of POAF, since by altering atrial conduction it facilitates re-entry, and predisposes to the subsequent development of POAF. We consider that extracorporeal circulation contains enough systemic inflammatory mediators that may be, in part, responsible for the occurrence of POAF. Similarly to other literature observations [16], we found some vascular changes, consisting of mildly decreased microcirculation in POAF, which associated with interstitial fibrosis especially in these ischemic patients.

Conclusions

In the present study, we examined the preoperative histological and immunohistochemical status of the atria in POAF patients in comparison with POSR ones. Our results suggest that preoperative morphological alterations, like increased size of CMs by vacuolation or hypertrophy and increased IF, may constitute a pathological substrate for POAF.

Our results allow us to consider that the various components of myocardial cells, such as α-SMA and desmin, and of non-cardiomyocytes, such as CD34 and CD68, are differently expressed under distinct ischemic conditions. Their expression varies in intensity and location in the severely ischemic POAF myocardium as compared to the mild ischemia from POSR heart.

Our study has some limitations: (1) First, the number of patients in the study is limited. Increasing the number of patients studied would lead to obtaining a more accurate data analysis. (2) Second, we sampled only the right atrial appendage, while the left atrial tissue was not investigated, which is one of the most critical regions for initiating and maintaining AF. In order to resolve this problem, experimental studies are needed to point out extensive atrial changes, including lesions of the left atrial appendage in POAF cases.

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