The Involvement of Epicardial Adiposity and Inflammation in Postoperatory Atrial Fibrilation - Immunohistochemical Qualitative and Quantitative Assessment

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Postoperatory atrial fibrillation (AF) is a common complication after cardiac surgery. Among others cardiac structural changes, epicardial adiposity (EA) and inflammation could be associated with increased cardiovascular risk, including AF. Because there are not enough studies on the association between epicardial fat and inflammation in AF, this paper assesses the association between structural changes in human right atrial appendages (RAA) in patients with and without AF with focus on EA and inflammation. RAA specimens from 20 coronary patients (ages 60 ± 10 years) were divided in 2 groups including patients with and without postoperatory AF. Histological, immunohistochemical (IMH) and morphometrical methods were used for assessing EA, myocardial fatty infiltration, epicardial inflammatory foci, cardiomyocytes size and vacuolation and extent of interstitial fibrosis. Atrial changes were found in most patients from both groups, having higher proportions in postoperatory AF patients. EA extent and myocardial fatty infiltration were twofold to threefold higher in patients with postoperatory AF and associated epicardial mononuclear inflammation. RAA structural changes can indicate the susceptibility to develop postoperatory AF. Currently, EA and inflammation have recently emerged as new independent AF risk factors. So, we proposed to study RAA structural changes, including EA, epicardial inflammatory foci in relation with postoperatory AF frequency.

Keywords: epicardial inflammation, epicardial adiposity, atrial fibrillation, atrial fibrosis, cardiac surgery

Atrial fibrillation (AF) is the most frequent cardiac arrhythmia in clinical practice and is often associated to profound structural alterations of the atrial myocardium.

The atrial substrate refers to the various structural changes of the atrial wall, which result in disorganization and loss of homogeneity of the atrial myocardium and formation of re-entry circuits [1]. Increased interstitial fibrosis (IF) has been observed having a role in the formation of local conduction blocks [2]. In addition to the IF, myocytes tend to enlarge due to replacement of their contractile apparatus by accumulation of glycogen or by compensatory hypertrophy [3].

A large research effort has been dedicated to identify new biomarkers and therapeutic targets for cardiac arrhythmia. Recently, a relationship between the thickness of epicardial adipose tissue (EAT) and the incidence and severity of AF has been reported [4]. Adipose tissue is a biologically active organ releasing adipokines. It is also a major source of cytokines. There is no distinct barrier between the EAT and the adjacent myocardium, supporting the possibility of crosstalk between the two tissues [5]. EAT accumulation is often associated with fatty infiltration from the epicardial layer, which advances deep into the myocardium. This may contribute to myocardium functional disorganization and formation of local arrhythmogenic substrate [6]. The infiltration of adipocytes into the atrial myocardium could also disorganize the depolarization wave front favoring micro re-entry circuits and local conduction block.

The discovery of a relationship between the abundance of atrial fatty deposits, epicardial inflammation and the risk and severity of AF, could open new research perspectives on the biology of the arrhythmogenic substrate.

Experimental part
Materials and methods
Approval to conduct the research was obtained from the institution's Board. The study included 20 patients hospitalized for coronary surgery in 2012, 14 men and 6 women aged between 36 and 74 years. All patients gave their consent to participate in the study prior to cardiopulmonary bypass surgery. We also had the Ethics Committee approval.

All patients were monitored daily until discharge with continuous electrocardiographic recording with standard 12-lead electrocardiography. Only AF episodes lasting longer than 15 min were considered. Patients were considered to have postoperatory atrial fibrillation (PAF) if interventional therapy (drugs or electrical cardioversion) was required to restore sinus rhythm.

The twenty coronary patients, 10 patients with PAF and 10 with postoperatory sinus rhythm (PSR), were selected by clinical criteria: absence of transitory PAF, no concomitant hyperthyroidism, and no valvular diseases. Tissue samples from the right atrial appendages (RAA) of the PAF group of patients were compared with samples from patients who remained in PSR.

Our study aimed to identify RAA structural particularities under ischemic conditions in association or not with PAF. The work was consisted in combining histopathological, immunohistochemical (IMH) and morphometric analysis.

Histological examination was done in accordance with current standard protocols for paraffin-processed tissues by using 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 and identification of RAA cellular and extracellular changes: cardiomyocyte (CM) hypertrophy and vacuolation, IF, epicardial adiposity (EA) and myocardial adipose tissue infiltration (MATI).

We also showed epicardial inflammatory foci presence that required IMH confirmation. Immunohistochemistry was applied according to standard protocols for paraffin-embedded tissues. IMH examination focused on inflammation assessing by using CD3 and CD68 markers.

Quantification of lesions was performed by morphometry using a color image analysis system: QuickPHOTO MICRO 3.0. The results were expressed only by means or percentages due to small study groups.

Myocytolysis, appearing as cytoplasmic vacuoles, was determined only in the CMs containing the nucleus in the cross section plane. Morphometric quantification of vacuolated CMs number was reported to the total CMs number visualized on a high power field (HPF has a magnification of x400).

CM size wad done by assessing CM diameters on transversely sectioned muscle fibers displaying the nucleus in the section plane. Hypertrophy means an increase in CM size. Hypertrophy morphometric quantification was done by referring the hypertrophic CM number to the total number of CMs on the studied HPFs.

IF, resulting by increasing of interstitial fibrous tissue (7), was quantified by reporting the fibrous interstitial area (stained in red with SR dye) to the entire histological section area on HPF.

EAT [7] is a visceral fat deposit of about 1.0 to 4.0 mm thickness, located between the myocardium and epicardium. It potentially causes epicardial inflammation. EAT measurement was assessed by reporting EAT area to the entire histological section area on HPF.

Microscopically, we analyzed 10 histological fields on HPFs for each case. In myolysis and hypertrophy, the results were expressed as percentage or mean values for the number of myolytic or hypertrophic cells referred to the total nucleated cell number. The degree of fibrosis and EAT was evaluated by relating IF or AT area to the entire studied histological section area on HPF. Results were expressed as percentage of the studied area related to the area of the entire histological section.

Epicardial inflammation (EI) is suspected to be an independent risk factor, a possible mechanism in local or systemic inflammation [8]. Inflammatory cell infiltration was investigated by IMH staining using antibodies against CD3 and CD68.

CD3 marker was used for identifying T lymphocytes, which normally are absent within a healthy epicardium. CD68 is a useful marker for macrophage lineage. Normal macrophage number varies significantly among patients (range 0 ± 6 cells/high power field-HPF). The precise amount of lymphocytes and macrophages within the diseased human heart is unknown.

Immunohistochemically we analyzed 10 histological sections at high magnification (x400 HPF) for each case. The results were expressed as percentage or mean values of the number of positive cells as related to the total number of nucleated cells in the studied area.

Results and discussions

From 20 selected patients aged from 36 to 74 years, with chronic myocardial ischemia, 10 were with PSR and 10 with PAF. Average age of patients with PAF was higher (64.77 years) than that of patients who remained in PSR after cardiac surgery (54.7 years). The paper associated histopathological, immunohistochemical, morphometric and statistical studies.

Histological structural atrial changes observed in both study groups were CM vacuolation, CM hypertrophy and interstitial fibrosis. EAT with myocardial AT infiltration and epicardial inflammatory foci were present, as well (fig. 1).
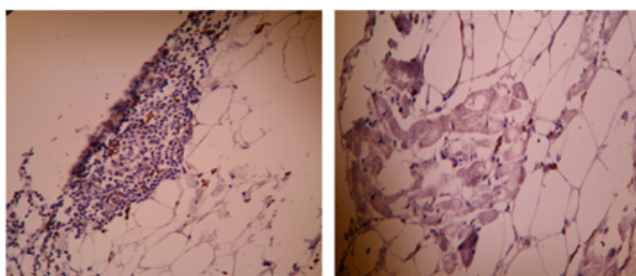

Fig. 1. Significant epicardial inflammatory focus related by EAT (a) and myocardial fatty infiltration (b) (CD3, CD68 x 20).

CM vacuolation attained various degrees in the two studied groups. In PSR, myolysis represented one fifth of the entire cell number (21.93%), while in PAF, CM vacuolation (fig. 2) was slightly higher (28.61%). In PSR, we found a uniform increase in CMs size without involvement of atrial architecture, while in PAF, we identified various size CMs with significant atrial architecture alteration.
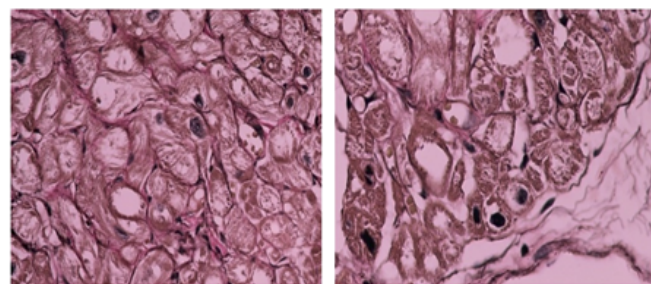

Fig. 2. Myocytolysis: (a) PSR, (b) PAF – vacuolated CM (EVG, x40)

CM hypertrophy (fig. 3) was observed in both PAF and PSR patient groups, although we have not registered differences between the two study groups (in PSR = 8.57%; in PAF = 9.07%).
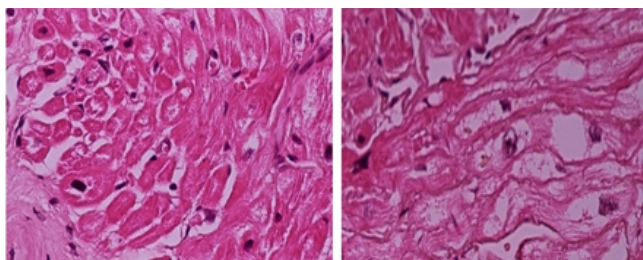

Fig. 3. CM hypertrophy: (a) PSR, (b) PAF – Hypertrophy and vacuolated CMs (HE, x40)

IF (fig. 4) was identified in both groups, having various degrees, with a higher proportion in PAF (23.41%) than PSR (16.76%). In PAF patients, we found larger collagenous bands separating large groups of CM cells, which could affect electrical conduction, while in patients with PSR a high degree of fibrosis was observed only in elderly patients. No interstitial inflammatory infiltrate was seen (data not shown).
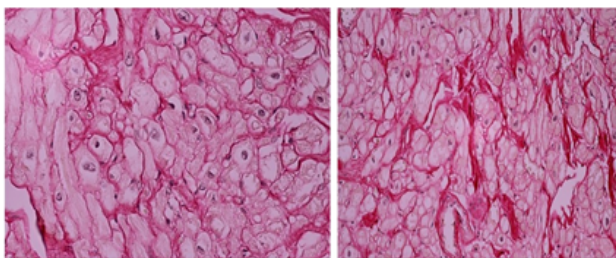

Fig. 4. Fibrosis: (a) PSR, (b) PAF – IF separating enlarged CMs (SR, x40)

EAT (fig. 5) is the adipose tissue accumulated between the visceral pericardium and the myocardium. Enlarged EAT area had a high frequency in PAF (68.03% of cases) than in PSR (22.94% of cases) registering a significant difference between the two groups.

The IMH study allowed accurate diagnosis of **EI** (fig. 5). The lymphocytic inflammatory foci within epicardial fat had high values in PAF (75.6 %) in comparison with PSR patients (21.33%), as well.
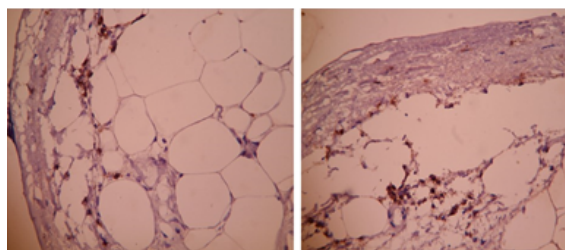

Fig. 5. Lymphocytic EI: (a) PSR, (b) PAF – epicardial lymphocytes (CD3, x40)

The epicardial inflammatory macrophage foci (fig. 6) related by epicardial adiposities registered a significant difference between the two groups (81.2 % in PAF; 18.8% in PSR), as well.
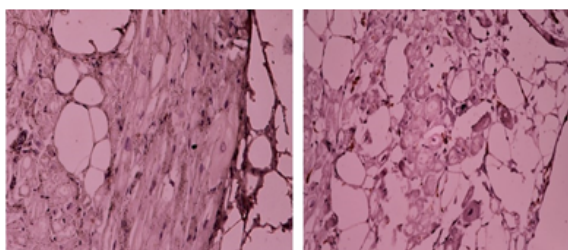

Fig. 6. Macrophage EI: (a) PSR, (b) PAF – Epicardial macrophages (CD68, x40)

As a consequence of epicardial adiposity expansion, myocardial infiltrating AT cells (fig. 7) separated the myocardial cells, with consequent atrophy of some of them. But CM atrophy was not a constant atrial feature in the two patient groups (data not shown).

The study indicated that patients with PAF developed deep structural changes in atrial epi-myocardium. Histological changes were noted in most specimens, generally showing more advanced damages in PAF patients than in PSR patients [9].

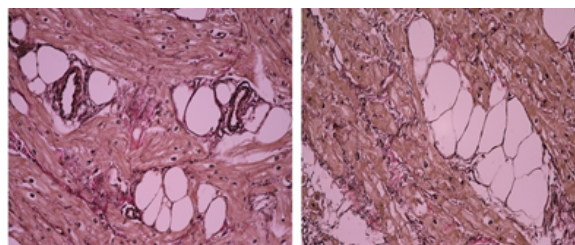Morphometric data showed remarkable differences between patient specimens with PAF and PSR, generally,

Fig. 7(a) PSR, (b) PAF – MATI and CM atrophy (EVG, x40)

having higher values in PAF patients comparative with PSR patients.

One of the most striking results of our study was the increased CM vacuolation in patients with PAF, reflecting CM exposure to hypoxic stimuli. Vacuolation was shown to occur during reversible myocardial damage and was suggested to be a predictor of AF development [10].

Another significant result of our study was the increased CM size, especially in patients with PAF. The CM hypertrophy, as an adaptive reaction to hypoxia, was twice as frequent in PAF patients as in the PSR ones. In a previous study, Tinica et al. [9] identified the CM HT as a histological predictive risk factor in AF.

Lubitz et al [11] showed that atrial fibrosis increases susceptibility to developing PAF. Our study group [3] considers fibrosis a sign of chronic myocardial ischemia causing decrease of the tissue conduction.

However, in this study we could not demonstrate any significant differences between patients with PSR and PAF in CM size, CM vacuolation and IF. We think that the absence of significant differences between the two study groups may be due to small size of patient groups.

Histologically, we did not find any atrial interstitial inflammation. The epicardial inflammatory foci were reduced in PSR and enhanced in PAF, probably reflecting local effects of the proinflammatory cytokines released from the epicardial adipose tissue [9].

In the last decade the interest in heart adiposity associated to consequent myocardial fat infiltration has renewed. According with Liu T [12], epicardial fat is a source of several proinflammatory cytokines. However, it also secretes adiponectin with anti-inflammatory effects, as well [13]. Regulation of local pro-inflammatory and anti-inflammatory balance in periatrial adipose tissue, together with anti-oxidants use, may be an important therapeutic target in the prevention of AF [14,15].

According to Iozzo P [6], EAT expansion and consequent myocardial fatty infiltration has an adverse lipotoxic, prothrombotic, and proinflammatory effect. Infiltrating fat may separate the myocardial cells, thereby reducing the number of sites of intercellular communications, causing a delay in the myocardial transmission of impulses, with the subsequent development of re-entrant arrhythmias.

Substantial evidence shows that an excess of certain free fatty acids can induce arrhythmias, as well [16]. We think that the excess of fatty acids can be counteracted by medication.

Conclusions

Our data indicate that epicardial fat measured by morphological tools seems to be associated with the presence and severity of AF. Measures of epicardial fat in larger samples in association with AF are needed to confirm these findings.

Regarding EAT, our findings suggest that inflammatory cell infiltration is enhanced in epicardial adipose tissue. We suppose that inflammation in epicardial fat may influence the pathogenesis of AF in terms of trigger factor for arrhythmic heart.

Finally, we think that epicardial adiposity and inflammation could be new markers of cardiovascular risk in AF and new therapeutical targets, as well.

Study limitations

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..

Second, it is not clear whether local inflammation alone is enough in AF development. To settle this issue, more studies are needed to focus on both systemic and localized inflammation in relation to EAT and AF persistence.

References

1.NATTEL, S., LI, D., YUE, L., Ann. Rev. Physiol., **62,** 2000, p. 51.
2.KOSTIN, S., KLEIN, G., SZALAY, Z., et al., Cardiovasc. Res., **54,** no. 2, 2002, p. 361.
3.BUTCOVAN, D., LUPUªORU, C.E., BARAN, D., et al., Rev. Chim. (Bucharest), **67,** no. 10, 2016, p. 2012.
4.HATEM, S.N., SANDERS, P., Cardiovasc. Res., **102,** no. 2, 2014, p. 205.
5.SACKS, H.S., FAIN, J.N., Am. Heart. J., **153,** no. 6, 2007, p. 907.
6.IOZZO, P., Diabetes Care, **34,** suppl. 2, 2011, p. S371.
7.BERTASO, A.G., BERTOL, D., DUNCAN, B.B., et al., Arq. Bras. Cardiol., **101,** no. 1, 2013, p. e18.
8.HIRATA, Y., KUROBE, H., AKAIKE, M., et al., Int. Heart J., **52,** no. 3, 2011, p. 139.
9.TINICA, G., MOCANU, V., ZUGUN-ELOAE, F., et al., Exp. Ther. Med., **10,** no. 6, 2015, p. 2299.
10.AD, N., SNIR, E., VIDNE, B.A., et al., Ann. Thorac. Surg., **72,** no. 3, 2001, p. 688.
11.LUBITZ, S.A., YIN, X., FONTES, J.D., et al., J.A.M.A., **304,** no. 20, 2010, p. 2263.
12.LIU, T., LI, G., J. Am. Coll. Cardiol., **57,** no. 10, 2011, p. 1249.
13.HATEM, S.N., Arch. Cardiovasc. Dis., **107,** no. 6-7, 2014, p. 349.
14.BOGATU, L., DRAGOMIR, R.E., ROSCA, P., Rev. Chim. (Bucharest), **67,** no. 1, 2016, p. 157.
15.NICOLAICIUC, O., MIHAI, C., SUFARU I.G., MARTU, I, SOLOMON, S.M., TATARCIUC, D,. BUDACU, C.,REZUS, C., MARTU, S., Rev. Chim. (Bucharest), **68,** no. 3, 2017, p. 620
16.SOLOFF, L.A., Am. Heart J., 80, no. 5, 1970, p. 671