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DOCTORAL THESIS

CORRELATION BETWEEN EPICARDIAL ADIPOSE TISSUE AND ATRIAL FIBRILLATION BURDEN IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFT SURGERY

S.S.D. MED/11

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ABSTRACT

Background: The epicardial adipose tissue is located between the myocardium and the visceral pericardium, lying directly above the myocardium without any fascia. Epicardial adipose tissue presents itself with histological features typical of the brown adipose tissue. It plays a cardioprotective role through thermoregulation, energy homeostasis and anti-inflammatory regulation. However, in pathological conditions, epicardial adipose tissue may have a pro-inflammatory effect. Less is known about the role played by epicardial adipose tissue in patients with a history of atrial fibrillation. Some studies suggest an association between increased epicardial adipose tissue (volume and thickness) and atrial fibrillation. Nonetheless, there is little data about histological characterisation of epicardial adipose tissue in patients with a history of atrial fibrillation.

Aim of the study: To evaluate the quantitative (using echocardiography) and qualitative characteristics (intra-operatory biopsy for histological characterisation) of epicardial adipose tissue in relation to atrial fibrillation burden after coronary artery bypass graft.

Patients and methods: Prospective single-centre study approved by the ethics committee of Verona and Rovigo in July 2018. Patients undergoing coronary artery bypass graft with preserved left ventricular ejection fraction were included, after giving informed consent. Patients with atrial fibrillation and immunosuppressive therapy history were excluded. All enrolled patients underwent a medical evaluation to collect clinical history, a transthoracic echocardiography to measure epicardial adipose tissue thickness and collection of a bioptic sample containing right appendage and epicardial adipose tissue during coronary artery bypass graft. After surgery post-surgical clinical course and telemetry were collected. Lastly, histological characterisation (PLIN1 and fibrosis) of the bioptic samples was performed.

Results: 56 patients undergoing coronary artery bypass graft were enrolled between 10th September 2018 and 3rd September 2019 in Cardiology and Cardiac Surgery departments. The mean hospitalisation was 11,9 ± 6,9 days and the postsurgical hospitalisation was 7,9 ± 3,7 days. 44 (78,6%) patients were male and

the median age was 68,45 ± 9,2 years. All patients were continuously monitored with telemetry from the day of cardiac surgery until discharge. No major complications occurred, only one death unrelated to the surgery. Out of the total number of patients, 22 (39%) had at least one episode of atrial fibrillation. In the population that developed atrial fibrillation there was a bigger atrial volume, a higher degree of diastolic disfunction (E/A rate), a thicker layer of epicardial adipose tissue and an older median age in comparison to the group that did not develop it. Epicardial adipose tissue measured using echocardiogram with a cut off of 4 mm was a predictor of atrial fibrillation with an OR of 1,49 [1,09-2,04], 73% of sensibility and 89% of specificity. Furthermore, from the histological analyses of biopsies, the patients with atrial fibrillation had a significantly higher percentage of fibrosis, while adipose infiltration was not significantly higher. Through univariate analysis, atrial volume (OR 1,05 CI 1,01-1,09, p 0,022), E/A rate (OR 0,04 CI 0,02-0,72 p 0,29), the percentage of fibrosis (OR 1,12 CI 1,00-1,25 p 0,045) and age (OR 1,17 CI 1,07-1,28 p 0,001) were predictors of atrial fibrillation as well as the thickness of the epicardial adipose tissue. Through multivariate analysis atrial volume (p 0,027), fibrosis (p 0,003) and age (p 0,039) were independent predictors of atrial fibrillation.

Conclusion: Post cardiac surgical atrial fibrillation is frequent. Epicardial adipose tissue measured by echocardiogram, atrial volume, fibrosis and age are predictors of post cardiac surgical atrial fibrillation.

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INTRODUCTION

Atrial Fibrillation

Atrial fibrillation is the most frequent sustained cardiac arrhythmia in humans with a prevalence of around 3% in the general adult population over 20 years of age. The incidence of atrial fibrillation increases progressively with age, from 0.5% in the 40-50 age group, to 5-15% beyond 80 years old, and affects men more frequently than women.[1] The prevalence of atrial fibrillation has increased steadily in recent decades, both as a result of the ageing of the general population and the improvement of diagnostic tools, and is expected to double over the next 50 years in Europe.[2] Atrial fibrillation is a potentially disabling arrhythmia. It leads to an increase in chance of death, thromboembolic events, heart failure and hospitalisations, negatively affects quality of life and ability to exercise, and lastly can cause left ventricular dysfunction. The most known and feared complication of atrial fibrillation is cardioembolic stroke, where risk increases by 5% compared to the general population and represents 20% of all causes of stroke. Cardioembolic stroke secondary to atrial fibrillation often also has a higher degree of clinical severity when compared to strokes of another origin, resulting in long-term disability or death.[3]

The cardioembolic risk in atrial fibrillation is conventionally defined by a risk score, that takes into account some important clinical features (CHA_2DS_2VASC - congestive heart failure, arterial hypertension, age >65-75 years, diabetes mellitus, previous cardioembolic event, vascular disease and female gender) and does not seem to be significantly influenced by the pattern and clinical features of arrhythmia manifestation.[4] The general treatment of atrial fibrillation should primarily aim at preventing thromboembolic events through appropriate anticoagulant therapy, then at alleviating symptoms, controlling heart rate and restoring sinus rhythm whenever possible. It is important to treat coexisting cardiovascular diseases that could play a pathogenic role in the origin and maintenance of the arrhythmia and increase the risk of complications related to atrial fibrillation.

Regarding the strategy of maintaining sinus rhythm, in addition to the traditional pharmacological approach which uses antiarrhythmic drugs, in recent years extensive development has found non-pharmacological strategies. One strategy in particular includes ablative techniques aimed at treating the substrate of atrial fibrillation. Atrial fibrillation ablation usually performed percutaneously and only rarely surgically, can use various sources of energy, the most common being radiofrequency and cryoenergy, and lastly laser source. These methods, when properly followed, have proven to be very effective in the treatment of the arrhythmia, in some cases resulting in a complete cure from recurrences.

From a pathophysiological point of view, the activation and perpetuation of a tachyarrhythmia require both a trigger and a substrate for its maintenance. Triggers play a key role in initiating the arrhythmia, in most cases the main location being the pulmonary veins. They can also be located in the posterior wall and, less frequently, in other sites in the left atrium. The mechanisms of arrhythmia maintenance require multiple re-entry circuits. This kind of electrophysiological mechanism requires the presence of a substrate, generally characterised by structural alterations of the atrial musculature. In the mechanism of multiple re-entry, the atrial fibrillation is perpetuated by the constant presence of a series of independent circuits. These propagate in an almost chaotic way inside the atrial wall with wave fronts that constantly interfere with each other, at times fragmenting and giving rise to further activation waves, at times blocking, colliding or merging and thus becoming numerically inferior. The predominance of substrate, trigger or focal activity accounts for different clinical types of atrial fibrillation. In focal atrial fibrillation, or lone atrial fibrillation, triggers and focal activity are the key factors in the origin of the arrhythmia, while in persistent or permanent atrial fibrillation the role of the substrate prevails over that of the triggers.

Atrial fibrillation from an electrocardiographic point of view is characterised by irregular RR intervals where a repetitive pattern is not recognisable, absence of P waves and atrial cycle duration <200 ms (>300 bt/min). Atrial fibrillation is diagnosed by recording a standard 12-lead electrocardiogram (ECG) or an ECG trace of at least 30 seconds.[3] Continuous monitoring of this arrhythmia for a

limited period of time can be done by systems such as 24-hour Holter ECG, hospital telemetry, or, in cases where more prolonged monitoring is needed, with external loop recorders or implantable loop recorders.

Based on the presentation, duration, and spontaneous termination of the episodes, five types of atrial fibrillation are traditionally distinguished. Paroxysmal in cases where the duration is less than 7 days, by spontaneous sinus rhythm cardioversion or induced cardioversion. It's defined persistent in forms lasting more than 7 days and permanent in those forms for which no rhythm control interventions are consensually undertaken by the doctor and by the patient.[3] Lastly, silent (asymptomatic) atrial fibrillation may have as its first manifestation a complication related to the arrhythmia itself (ischemic stroke or tachycardiomyopathy) or may be diagnosed following a chance ECG. It may present itself as any of the temporal forms of atrial fibrillation described above. Continuous monitoring systems can be useful in defining the atrial fibrillation burden, which is defined as the total time the patient spends in arrhythmia regardless of the number of episodes or mode of presentation.

Atrial fibrillation and cardiac surgery

Postoperative atrial fibrillation is a common occurrence that can affect 20 to 50% of patients undergoing cardiac surgery (about 30% after coronary artery by-pass graft, 40% after valvular surgery and 50% after combined by-pass and valvular surgery).[5,6] These numbers are expected to increase in the coming years as the average age of patients undergoing cardiac surgery increases, considering that the incidence of this arrhythmia is strongly correlated with increasing age. The peak of the post-operative arrhythmic incidence is between the 2nd and 4th day after surgery, particularly on the second day.[6] Although this arrhythmia is generally well tolerated and considered a temporary problem related to the surgery, it is associated with worse clinical outcomes in the short and long term, leading to an increased risk of death, hospitalisation and complications such as stroke, heart failure and infections.[7,8] In particular, the risk of postoperative and perioperative stroke is about 3 times higher in patients with episodes of atrial fibrillation after cardiac surgery. From a financial standpoint, the onset of atrial fibrillation in the

postoperative cardiac surgery period can prolong hospitalisation times, impacting patient management costs.[9]

From a pathophysiological point of view the mechanisms of appearance and maintenance of postoperative atrial fibrillation are not yet fully known. It is likely that this arrhythmia requires the presence of an electrophysiological substrate that allows the propagation of multiple re-entry waves resulting from a dispersion of atrial refractoriness. The latter requires a structural atrial substrate, which could be a consequence of the association of several factors predisposing the arrhythmia itself, such as advanced age, arterial hypertension, diabetes mellitus, obesity, atrial enlargement, and factors related to the cardiac surgery itself. Some perioperative causative mechanisms have been proposed that include pericardial inflammation, excessive production of catecholamines, autonomic imbalance during the postoperative period, and interstitial mobilization of fluid with resultant changes in volume, pressure, and neurohumoral environment. These factors might alter the atrial refractoriness and slow atrial conduction.[6] Finally, inflammation and oxidative stress could play an important role in this scenario, as well as pericardial fat. Atrial fibrosis, which is the result of an inflammatory process and of metabolic disorder, is considered a key element of atrial remodelling. Numerous clinical and experimental studies in recent years have suggested that pericardial fat, such as atrial fibrosis, is strongly associated with the incidence, maintenance and recurrence of atrial fibrillation.

A greater understanding of the causes of this arrhythmia could help in the prevention and treatment after the onset of postoperative atrial fibrillation.

Cardiac adipose tissue

The heart is surrounded by a variable amount of perivisceral adipose tissue, visible on its surface. This manifests on the atrioventricular grooves, the posterior part of the atria, on the appendages and along the larger coronary arteries. Under physiological conditions it represents about 20% of the heart mass. It consists of a paracardial component, placed outside of the visceral pericardium, and an epicardial component placed between the visceral pericardium and epicardium, and combined they make pericardial fat (Figure 1). The epicardial and paracardial

adipose tissue have different embryonic origins and different biological properties. The epicardium consists of a population of mesothelial cells originating from the transverse septum area which migrate to the surface of the heart. The epicardial fat originates from the splanchnopleuric mesoderm similarly to mesenteric and omental fat, while the paracardial fat originates from the primitive thoracic mesenchyme. For this reason, the vascularisation is also different between the two types of fat. Coronary circulation supplies epicardial fat, while non-coronary circulation supplies paracardial fat.[10] A further anatomical distinction in regard to epicardial fat can be made between myocardial and pericoronary components. The latter directly surrounds the coronaries on the adventitia, while the myocardial component is in direct contact with the myocardial muscle itself. Whether there are functional differences between these two components is unclear.

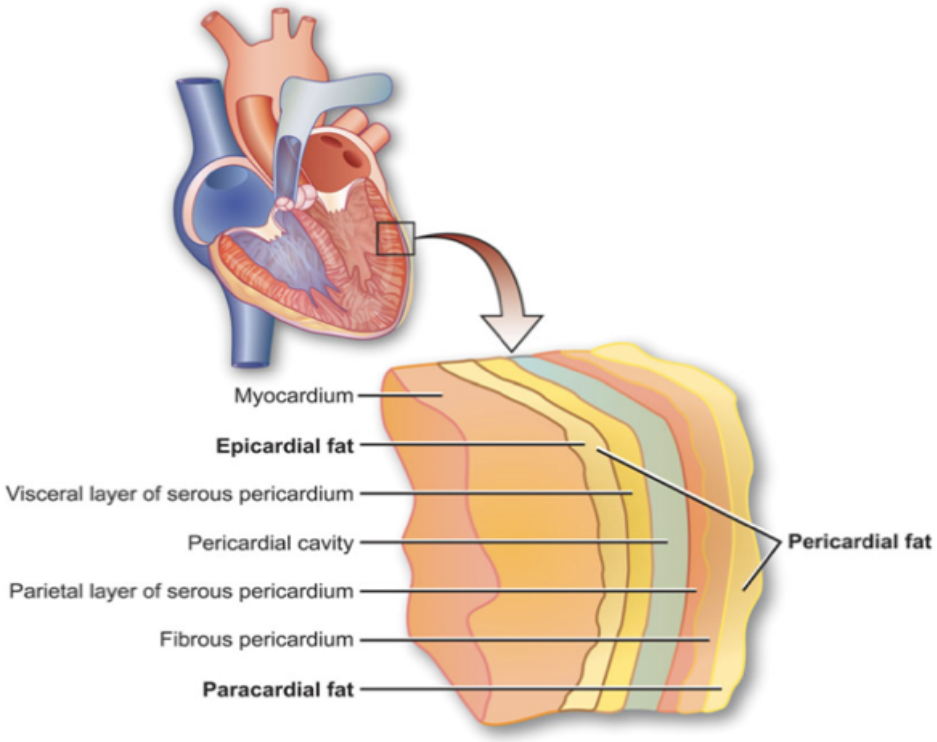

Figure 1: *The structure of pericardial fat consisting of a paracardial component located outside of the visceral pericardium and an epicardial component located between the visceral pericardium and epicardium.*

The properties of epicardial adipose tissue

Epicardial adipose tissue is in direct contact with the adjacent myocardium without the interposition of any barrier. Both these structures share the same microcirculation (Figure 2). The lack of an anatomic barrier between adipose tissue and myocardium allows paracrine activity between the two tissues, also favoured by a dense network of vasa-vasorum. Epicardial fat is a biologically active organ and expresses uncoupling Protein-1 (UCP-1), a protein of the inner mitochondrial membrane, which typically characterises brown adipose tissue.[11] It has a high capacity for free fatty acid release and low glucose consumption (higher capacity than other adipose tissues). Its biochemical properties suggest a role in providing the myocardium with energy and protection from hypothermia.

Lastly, epicardial adipose tissue produces numerous cytokines and adipokines that affect the surrounding tissue. These same properties under pathological conditions may promote paracrine and vasocrine secretion of pro-inflammatory cytokines. What balances this equilibrium is still unclear. Both the quantity and the biological activities of this tissue vary from subject to subject. Local vascularisation, weight loss and targeted drug therapy could promote the physiological properties of epicardial fat. In subjects with coronary heart disease, less anti-atherogenic adipokines and less anti-inflammatory cytokines are secreted in favour of more tumor necrosis factor (TNF) alpha, Interleukin-6 and chemokines such as monocyte chemoattractant protein-1 (MCP-1).[12,13,14] In addition, epicardial adipose tissue may contribute to myocardial insulin resistance by secreting TNF-alpha, which inhibits the insulin receptor and increases the release of non-esterified fatty acids.

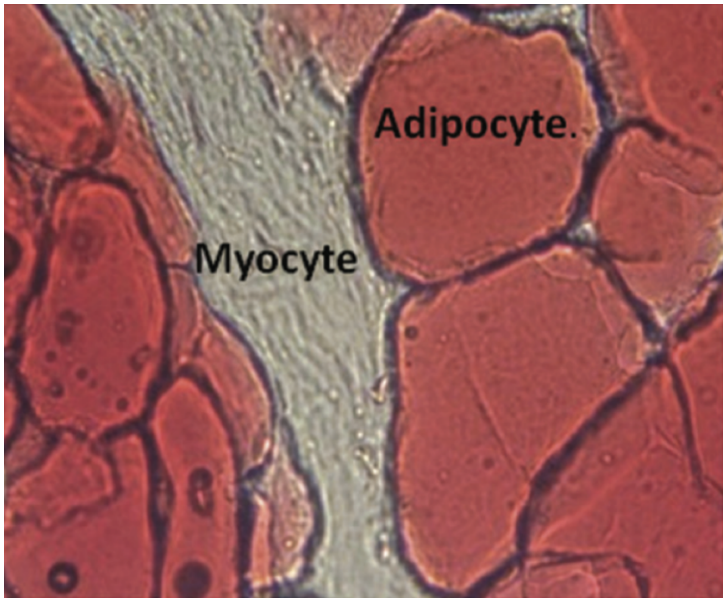

Figure 2: *Epicardial adipose tissue in direct contact with the adjacent myocardium without the interposition of any anatomical barrier. The lack of barrier between epicardial adipose tissue and myocardium allows paracrine activity between the two tissues.*

Intracellular adipose infiltration

Epicardial adipose tissue could play a pathological role not only through paracrine activity, but also through direct local infiltration between myocardial cells. In turn this could promote fibrosis of the extracellular matrix, and within them the so-called myocardial steatosis. It is well known that excess fatty acids are sequestered as triglycerides and stored in adipocytes or as lipid droplets. This phenomenon also occurs in tissues other than adipose, such as the liver, the pancreas, skeletal muscle and the heart. Some scientific data suggest that cardiac steatosis may have harmful effects on cardiomyocyte function regardless of the effects that may result from increased generalised adiposity or high blood lipid levels.[15]

Adipose tissue and atrial fibrillation

In recent years the association between epicardial and paracardial adipose tissue and atrial fibrillation has been studied, highlighting a possible role of these tissues in the onset and maintenance of arrhythmia, and in the recurrences after

percutaneous ablation with both radiofrequency[16] and cryoablation.[17] This correlation would depend both on paracrine properties, similar to those described above for coronary artery disease, and on direct adipose infiltration between the myocardial fibres. The latter would be able to promote atrial remodelling typical of atrial fibrillation and change myocardial electrical properties causing a slowdown of atrial conduction and thus favouring mechanisms of re-entry.

Most studies have used non-invasive imaging methods to study cardiac fat, without making a clear distinction between epicardial and paracardial tissue. A recent animal study compared a group of 10 sheep on a hypercaloric diet that made them obese, to a group of 10 sheep on a normocaloric diet.[18] The analysis of the atrial substrate for atrial fibrillation was carried out in both groups. The obese sheep group showed greater atrial vulnerability to the onset of atrial fibrillation and a greater burden. This group compared to the normal-weight sheep group had a higher fat infiltration histologically at the atrial myocardial level, and a higher percentage of atrial fibrosis and expression of tumor growth factor (TGF) beta.

Aims of the study

There has been little research of intra-operative epicardial fat in the context of atrial fibrillation. The objective of this study is to investigate the correlation between epicardial fat and atrial fibrillation in a postoperative context of patients undergoing cardiac surgery both through imaging and histological analysis of fat taken during cardiac surgery.

MATERIALS AND METHODS

Study design

An experimental monocentric study with no use of drugs or devices was conducted, aimed at assessing the role of epicardial fat in the pathogenesis of atrial fibrillation by echocardiographic and histological analysis of pericardial fat. Figure 3 summarises the design of the study.

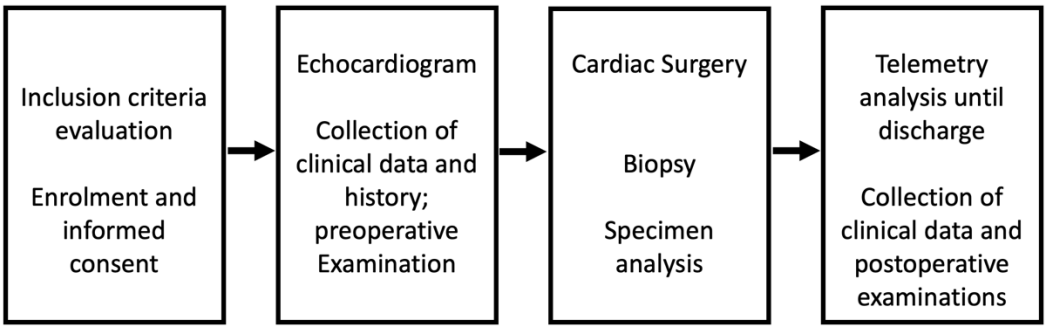

Figure 3*: Study design.*

Definition of atrial fibrillation burden

Atrial fibrillation burden is defined as the amount of time a person spends in atrial fibrillation, quantifiable by the number of episodes of arrhythmia and the duration of each episode.

Study objectives

Primary objective: To study the relationship between pericardial fat in its epicardial and paracardial components (measured by a preoperative echocardiogram) and atrial fibrillation burden in the immediate postoperative period.

Secondary objectives: Histological typing of fibrosis and of lipid infiltration of epicardial fat in relation to atrial fibrillation burden in the immediate postoperative period. Study of the correlation between epicardial and paracardial

fat and preoperative echocardiographic parameters predictive of atrial fibrillation, such as atrial strain, atrial size and diastolic function.

Study end points

Primary end point: Burden of atrial fibrillation in the immediate postoperative period in relation to epicardial fat measured in mm, by a preoperative echocardiogram with a long axis parasternal view.

Secondary end points: Burden of atrial fibrillation in the immediate postoperative period in correlation with the percentage of extracellular lipid markers known as Perilipin 1 (PLIN1) in the biopsy collection, percentage of fibrosis in the perimyocardial biopsy collection, atrial strain, atrial size in ml and degree of diastolic dysfunction.

Study population

The study was approved by the CESC Ethics Committee of the Provinces of Verona and Rovigo, N. Prot. 1744 CESC, on 26th July 2018. The enrolments were carried out for 12 months involving patients belonging to the 'Complex Operating Unit of Cardiovascular Diseases and Cardiac Surgery of the Integrated University Hospital of Verona' (AOUI Verona), who met the criteria of inclusion and exclusion of the study.

Inclusion criteria

- Candidates for cardiac surgery of coronary artery bypass surgery with ischemic heart disease and preserved ejection fraction;
- Sex: male and female;
- Age: adult patients able to express direct informed consent over 18 years old;
- Patients without a history of atrial fibrillation;
- Adhesion to the informed consent.

Exclusion criteria

- Pregnancy status;

- Patients unable to provide informed consent;
- Candidates for valvular surgery;
- Myocardial contractility deficit quantifiable by echocardiogram ejection fraction (less than 50%);
- Patients with known autoimmune or auto-inflammatory diseases;
- Patients with chronic atrial fibrillation;
- Patients with atrial fibrillation at enrolment.

Enrolment and preoperative evaluation

During the hospitalisation for cardiac surgery, candidates for coronary artery bypass surgery were evaluated. After verification of the inclusion and exclusion criteria, eligible patients were enrolled. Each patient was booked in after signing informed consent. Demographic and clinical-anamnestic information such as age, weight and height, cardiovascular risk factors, history of cardiovascular diseases, coronarographic data and any ongoing medical treatment before surgery was collected from each patient. In addition, clinical data were collected such as basal haematochemical values, in particular blood counts, electrolytes, liver function, kidney function, thyroid function, glycolipid function, glycated haemoglobin, uricemia, C reactive protein (CRP), procalcitonin (PCT) and fibrinogen.

An electrocardiogram was also performed for each patient to evaluate sinus rhythm. Furthermore, a complete echocardiogram was performed to measure epi and paracardic fat, particularly in long axis parasternal view at the aortic root, and in short axis. It included moreover measurement of atrial and ventricular strain, systolic function, diastolic function and atrial size. The choice of an ultrasonography as the imaging method for the evaluation of pericardial fat was dictated by the fact that the echocardiogram is an appropriate tool in predicting the correlation between the amount of pericardial fat measured and the burden of atrial fibrillation in different populations studied. In addition, it is a routine preoperative exam for the enrolled patients, while the use of other methods such as a computed tomography (CT) sca or magnetic resonance would have increased costs and length of hospital stay and might have required the administration of contrast medium in certain cases.

Biopsy collection and immunohistochemical analysis

Each biopsy was performed by the cardiac surgeon during surgery. The collection included waste tissue from the surgery, in particular a specimen of the right atrial appendage containing epicardial fat. The biopsy samples taken in the operating room were collected in a test tube containing 10% neutral buffered formalin and then stored in a 4°C temperature refrigerator until the analysis. The latter was carried out by expert personnel from the 'University Laboratory of Medical Research' (LURM) at 'Policlinico G. Rossi' in Verona.

At the LURM centre, analyses were performed to determine the degree of adipose infiltration and fibrosis on the right appendage specimen. Fibrosis in myocardium samples under examination was evaluated by Masson trichromic staining with nuclear staining (with Weigert's ferric hematoxylin) and connective staining (with aniline blue). The full biopsy preparations were analysed using the PluginColourDeconvolution with 100x magnification images, which divided the fibrosis zones in blue and the muscle zones in red in two different images. Fibrosis was calculated as the percentage of blue fibres among cardiomyocytes in relation to the total area determined by the sum of blue fibres and muscle in red (% = total blue area *100/; total blue area+red).

Specimens were obtained from atrial biopsies to determine lipid infiltration of the myocardium as adipocytes present between the cardiomyocytes using the PLIN 1 marker. The specimens obtained were observed using an Olympus BX51 optical microscope equipped with a KY-F58 CCD (JVC) camera. The images were captured using Image-ProPlus (software version 7. 0). Image analysis and quantification was performed using ImageJ (software version 1. 49s). The biopsy sections obtained were incubated with rabbit PLIN1 primary anti-human antibodies specific for marking adipocytes. The degree of adipocyte infiltration was expressed as the number of adipocytes marked with PLIN1 per square millimetre.

Postoperative assessments

The burden of atrial fibrillation in patients who developed this in the immediate postoperative period was assessed by telemetry analysis both in intensive care unit and in the cardiac surgery ward from the day of surgery until discharge. Both the

total duration of telemetric monitoring of individual postoperative patients and the number of episodes of atrial fibrillation with the relative duration of each episode were quantified. ECG traces were also collected.

Data were collected on postoperative clinical progress, in particular the requirement of cardiac inotropic drugs, postoperative anaemia requiring hemotransfusion, increase in inflammatory markers, need for chest drainage, duration of hospitalisation, other possible complications or death. Lastly, the pre-discharge basal blood chemistry values and the therapy with which each patient was discharged were noted from the medical records.

Statistical analysis

The estimate of the population to be recruited to achieve significance was calculated using the statistical program PASS 14. 0. 8 and the Logistic Regression module. The data collected was summarised using elements of descriptive statistics. The continuous variables are reported as average ± standard deviation (SD), the categorical variables are expressed as a percentage of the total. The comparison between groups was made by averaging the Student t-test for unpaired data. For paired data the comparison between groups was done by Chi Square analysis. Logistic regression was applied to analyse the predictors of an atrial fibrillation event. For the analysis of independent predictors, the MANOVA analysis was used. ROC analysis was applied to test the predictive value and the sensitivity and specificity of epicardial fat at a given cut-off in predicting events. This predictive power was finally tested with a square Chi test. The level of statistical significance was set at 5%. The statistical analyses were conducted using the IBM SPSS Statistics program, 2 Version 20. 0. (Armonk, NY, USA: IBM Corp.).

RESULTS

Population studied

56 patients were enrolled between 10th September 2018 and 3rd September 2019. These patients belonged to the 'Complex Operating Unit of Cardiovascular Diseases and Cardiac Surgery of the AOUI Verona' and were candidates for coronary artery bypass surgery. Enrolled patients entered an elective hospitalisation regime for cardiac surgery or an emergency regimen for recent onset angina with an indication for cardiac surgery treatment. The average stay was 11.9 ± 6.9 days, while the postoperative course from the day of surgery was 7.9 ± 3.7 days.

Out of 56 patients, in addition to the coronary artery bypass surgery, one case underwent aortic valve replacement for intraoperative valve mass finding and another underwent interatrial defect closure. Out of the enrolled patients 44 (78.6%) were male, with an average age of 68.45 ± 9.2 years. All patients were continuously monitored with telemetry from the day of cardiac surgery both in the intensive care unit and in the cardiac surgery ward until discharge. With regard to postoperative complications, most minor complications were resolved with minor interventions as summarised in Table I. One patient underwent coronarography for venous by-pass closure with good results. Another patient died from relapsing and severe gastrointestinal anaemia, unrelated to the recent cardiac surgery.

Hospital Stay and Post-surgical Course N=56	
Variables	**Value**
Length of average Hospital Stay (days ± SD)	11.9 ± 6.9
Length of average Post-surgical Course (days ± SD)	7.9 ± 3.7
Elective Cardiac Surgery (n/N %)	33 (58.9)
Transfusion-needing anemization (n/N %)	30 (53.6)
Thoracentesis-needing Pleural Effusion (n/N %)	10 (17.8)
Increase of inflammation markers (n/N %)	9 (16.1)
Indication of postoperative CGF (n/N %)	1 (1.8)
Death (n/N %)	1 (1.8)

Table I: *Hospital Stay and Post-surgical Course-related Variables. CGF: coronarography*

Postoperative atrial fibrillation

Of the 56 patients enrolled, 22 (39.3%) had at least one episode of postoperative atrial fibrillation. Details about the population who had postoperative atrial fibrillation are summarised in Table II.

AF Patients N=22	
Variables	**Valore**
Average Duration of post-surgical telemetrics (hours ± SD)	190.3 ± 89.8
Recorded AF episodes (n ± SD)	1.5 ± 0.8
AF burden (hours ± SD)	43.8 ± 39.5
Administration of amiodarone for AF treatment n/N (%)	22/22 (100)
Electrical Cardioversion n/N (%)	1/22 (4.5)
Discharge in sinus rhythm n/N (%)	21/22 (95.5)

Table II: *Data concerning patients affected by atrial fibrillation after surgery. AF: atrial fibrillation*

The comparison of the general characteristics of the population that had atrial fibrillation with the population that did not have it is shown in Table III.

AF vs. non-AF patients			
Clinical variables	**Non-AF (N=34)**	**AF (N=22)**	**P value**
Age (years ± SD)	64.5 ± 8.5	74.1 ± 6.7	<0.001**
Male n/N (%)	28 (84.8)	15 (68.2)	0.188
BMI (n ± SD)	27.2 ± 4.3	27.2 ± 4.0	0.941
Hypertension n/N (%)	24 (72.7)	16 (72.7)	1.000
Dyslipidemia n/N (%)	19 (57.6)	10 (45.5)	0.420
Pre-admission statin	24 (70.6)	17 (77.3)	0.581
Pre-admission beta blockers	19 (55.9)	17 (77.3)	0.103
Chronic Renal Failure n/N (%)	4 (12.1)	4 (18.2)	0.700
Tobacco Smoking n/N (%)	4 (12.1)	4 (18.2)	0.700
Diabetes n/N (%)	10 (30.3)	7 (31.8)	1.000
Peripheral vascular disease n/N (%)	32 (97.0)	21 (95.5)	0.380
ACS at admission n/N (%)	15 (44.1)	8 (36.4)	0.565
EF (n ± SD)	56.3 ± 4.5	55.8 ± 5.8	0.690
Atrial Volume (ml ± SD)	51.9 ± 13.4	64.2 ± 19.5	0.015**
TAPSE (mm ± SD)	24.2 ± 3.9	22.8 ± 3.9	0.268
E/A rate (n ± SD)	0.93 ± 0.24	0.77 ± 0.22	0.021**
E/E' rate (n ± SD)	8.8 ± 3.6	11.9 ± 5.6	0.070
Epicardial fat thickness (mm ± SD)	2.7 ± 1.9	4.1 ± 1.7	0.006**
Paracardiac fat thickness (mm ± SD)	4.9 ± 3.0	5.4 ± 2.1	0.571
Pericardial fat thickness (mm ± SD)	7.6 ± 3.5	9.5 ± 3.0	0,064
MAPSE (mm ± SD)	10.3 ± 2.8	11.7 ± 2.3	0.240
Surgery duration (min ± SD)	286.6 ± 44.7	291.0 ± 65.1	0.781
Aortic balloon pump n/N (%)	5 (15.2)	1 (4.5)	0.384
Treated vessels (n ± SD)	2.6 ± 0.9	2.2 ± 0.7	0.102
Inotropics use n/N (%)	15 (44.1)	15 (68.2)	0.097
Transfusion-needing anemization n/N (%)	17 (51.5)	12 (54.5)	1.000
Post-surgical course duration (days ± SD)	7.9 ± 4.0	8.1 ± 3.5	0.818
Post-surgical troponin peak (ng/L ± SD)	6993 ± 9227	4676 ± 5117	0.289
Percentage of fibrosis (% ± SD)	14.5 ± 6.4	19.5 ± 6.8	0.033**
Adipocites infiltration (n/mm ± SD)	1.5 ± 5.3	1.4 ± 2.1	0.926

Table III: *Comparison of variables between populations that developed atrial fibrillation and those that did not develop arrhythmia. AF: atrial fibrillation, BMI:*

body mass index, ACS: acute coronary syndrome, EF: ejection fraction, TAPSE: tricuspid annular plane systolic excursion, MAPSE: mitral annular plane systolic excursion.

The population that developed atrial fibrillation had some important atrial parameters significantly different in comparison to the population that did not develop arrhythmia, in particular a higher atrial volume, a higher degree of diastolic disfunction expressed by the E/A wave ratio and a higher epicardial fat thickness measured on the echocardiogram. This population also had a significantly higher average age. Laboratory analysis showed that patients with atrial fibrillation had a significantly higher percentage of fibrosis in the biopsy, while the comparison of adipocyte infiltration between the two populations was not significant (Figure 4). The remaining parameters compared were not significantly different between the two populations, in particular the male gender, body mass index, arterial hypertension, dyslipidemia, total blood cholesterol, statin therapy before admission, chronic renal failure, tobacco smoke, diabetes mellitus, peripheral vasculopathy, acute coronary syndrome at admission, ejection fraction, TAPSE (tricuspid annular pla-ne systolic excursion), MAPSE (mitral annular plane systolic excursion), E/E' ratio, paracardial and pericardial fat thickness.

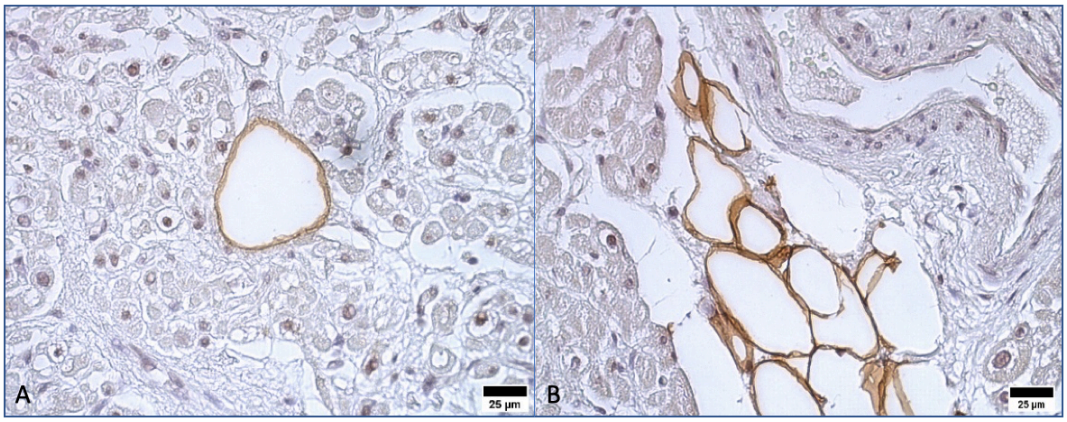

Figure 4*: Significant images of PLIN1(+) adipocytes between the cardiomyocytes (bar scales = 25µm) of two patients in the study.* **A** *patient who didn't develop postoperative atrial fibrillation.* **B** *Patient who developed postoperative atrial fibrillation.*

The variables found to be significantly different in the two populations were subsequently subjected to univariate analysis for the study of the variables associated with the onset of atrial fibrillation in the postoperative period. Finally, all variables tested in the univariate analysis were subjected to MANOVA analysis to test the predictive value of these variables independently. Among these atrial size, atrial fibrosis and age were found to be independent predictors of postoperative atrial fibrillation (Table IV).

Clinical Variables	**OR**	**95% CI**	**P value**
Univariate Analysis – Logistic Regression			
Age	1.17	1.07-1.28	0.001
Atrial Volume	1.05	1.01-1.09	0.022
E/A rate	0.04	0.02-0.72	0.029
Epicardial Fat	1.49	1.09-2.04	0.012
Atrial Fibrosis (percentage)	1.12	1.00-1.25	0.045
Multivariate Analysis – MANOVA			
Atrial Volume			0.027
Atrial Fibrosis			0.003
Age			0.039

Table IV: *Univariate and Multivariate Analysis on variables related to atrial fibrillation onset after cardiac surgery.*

Primary endpoint

The analysis of the primary endpoint from the data shows a significant difference in epicardial fat between the two populations that either developed or did not develop postoperative atrial fibrillation. Epicardial fat has an Odds Ratio of 1.49 [1.09-2.04] in predicting the onset of postoperative atrial fibrillation. When tested, the predictive value in a ROC analysis, the area under the curve (AUC) has a value of 0.73 ($p=0.004$), and a cut-off of 4 mm has a 73% sensitivity and 89% specificity in predicting the event (Figure 5). The predictive power of the 4 mm cut-off was also tested with a square Chi test that confirms its validity as a predictive value ($p<0.001$).

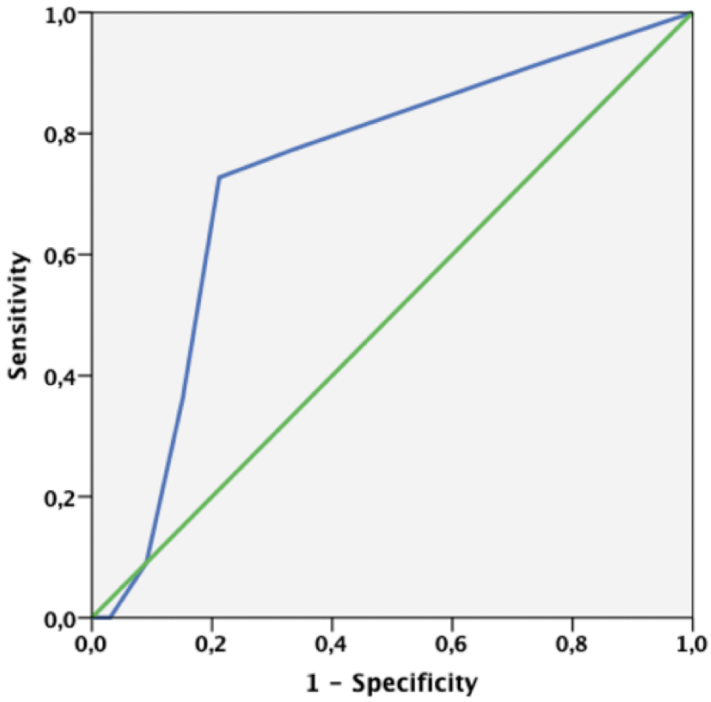

Figure 5*: ROC curve. The area under the curve (AUC) has a value of 0. 73 (p=0. 004), and a cut-off of 4 mm has a 73% sensitivity and 89% specificity in predicting the atrial fibrillation event.*

DISCUSSION

In this study, the percentage of patients who developed atrial fibrillation is about 39%, in line with literature data.[5-9] It appears to be a strong predictor of atrial fibrillation independently of other factors such as age, which is also confirmed by several studies.[1,2]

With regard to the analysis of atrial fibrillation burden it is important to underline that in this study all patients were monitored continuously with telemetry from the day of surgery until the day of discharge with a precise definition of the arrhythmic burden. In postoperative atrial fibrillation prophylaxis, although our data do not show a statistically significant difference between populations, 36 (64.28%) patients who underwent surgery were on a beta-blocker therapy prior to surgery. 17 of these were in the group of patients who developed atrial fibrillation. None of them were using a Sotalol or Cordarone treatment before the surgery. All patients also had a temporary epicardial pacemaker with atrial and/or ventricular stimulation in case of bradycardization and/or conduction blockages which in some studies has shown to be associated with a lower incidence of postoperative atrial fibrillation.[19] Pharmacological treatment of episodes of atrial fibrillation in the postoperative period was carried out according to current guidelines[3] with the administration of amiodarone ev which proved effective in 20 cases out of 22. In one case it was necessary to proceed with electrical cardioversion and in another case with the strategy of rate control with the choice of a late programmed electrical cardioversion. A systematic review of 58 studies with a total of 8565 patients showed that interventions to prevent and/or treat postoperative atrial fibrillation with beta blockers, sotalol or amiodarone and, to a lesser extent, by atrial stimulation have a favourable impact on the outcome of cardiac surgery patients (atrial fibrillation, stroke, and length of hospital stay- OR 0.43; CI 95% 0.37-0.51).[19]

Of particular relevance in this project is the study of epicardial fat through cardiac surgery. There are numerous studies on epicardial fat in the animal model, while in humans there are few, all in the context of cardiac surgery. The reason is the difficulty to access epicardial fat without undergoing an invasive procedure.

Hiroko Kogo et al.[20] conducted a study on 77 patients undergoing both valvular and myocardial revascularization cardiac surgery, after measuring epicardial fat at the chest CT scan. While the latter parameter was associated with atrial fibrillation burden, histological analyses performed on biopsy samples did not show a significant correlation between fibrosis and adipose infiltration and atrial fibrillation burden. The Qing Wang group conducted research on the inflammatory pattern in epicardial fat and atrial fibrillation in patients undergoing heart surgery. In particular, in a group of 89 patients in postoperative sinus rhythm, both ischemic and valvular, the expression of activin A in epicardial fat, an atrial fibrosis marker, was an independent predictor of postoperative atrial fibrillation.[21] In the other study of 64 patients undergoing surgical myocardial revascularization (including 28 patients in atrial fibrillation and 36 in sinus rhythm) the presence of YKL40 in epicardial fat, an inflammatory remodelling and fibrosis marker, was significantly higher in patients in atrial fibrillation, as well as the percentage of fibrosis found in the same specimen.[22]

In this study emerged relevant data regarding pericardial fat and the onset of postoperative atrial fibrillation. This could help understand the pathophysiological role of this tissue in promoting the creation of a substrate for arrhythmia.
The relevant points are:

- Epicardial fat, and not paracardial fat, is significantly associated with the onset of arrhythmia and is a predictor of the event.
- Adipose infiltration does not appear to be significantly associated with atrial fibrillation.
- Fibrosis is significantly higher in the population that developed atrial fibrillation and is an event predictor independent of other factors.
- Some parameters that reflect diastolic dysfunction are related to the onset of atrial fibrillation, including atrial size and E/A ratio.

Epicardial fat

In the enrolled patients, the thickness of epicardial fat was significantly higher in those who developed atrial fibrillation in the postoperative period. In particular a

cut-off of 4 mm epicardial fat had a 73% sensitivity and 89% specificity in predicting the event. There was also a difference between epicardial fat and paracardial fat in relation to the development of atrial fibrillation, because the latter does not seem to be significantly related to the event under analysis. A similar figure is reported by several imaging studies. Tze Fa Chao et al.[23] a pre-procedural echocardiogram with measurement of epicardial fat thickness in long axis parasternal view was performed in 283 patients undergoing transcatheter ablation of atrial fibrillation, similarly to what was evaluated in this study. The average thickness in the population was 6.1 ± 0.8 mm with a thickness of 7 ± 0.7 in patients with persistent forms compared to patients with paroxysmal forms of atrial fibrillation with 5.9 ± 0.7. Atrial volume and epicardial fat thickness were independent predictors of atrial fibrillation recurrence in the follow-up at 16 ± 9 months. Alexander R. van Rosendael et al.[24] quantified posterior atrial wall adipose tissue in 400 patients undergoing chest CT scans, 200 with atrial fibrillation and 200 with no history of arrhythmia. The adipose tissue was significantly more represented in the atrial fibrillation group than the sinus rhythm group (10.6 ± 5.5 versus 4.7 ± 3.5 g, P<;0.001) and each additional gram of fat was a predictor of atrial fibrillation with an odds ratio of 1.32. In this paper, however, there was no clear distinction between epicardial and paracardial fat, but the entire pericardial fat was assessed in a different location from that assessed by the current research. In these studies, moreover, there were no histological data on direct myocardial infiltration and atrial fibrosis.

An important consideration with regard to the increased amount of epicardial fat in the population developing postoperative atrial fibrillation is the clinical impact that could result from the reduction of this tissue. In fact, assuming that a greater amount of epicardial fat can promote the manifestation of the arrhythmia by contributing to the formation of an arrhythmogenic substrate, measures aimed at reducing epicardial fat through weight loss, could reduce the arrhythmogen risk (in this case considering overweight as a surrogate for a greater amount of epicardial fat). In this regard, several studies in the literature show a correlation between obesity and increased burden of atrial fibrillation in different populations, and conversely, a reduction in atrial fibrillation in patients who experience weight

loss in situations of overweight.[25] However, in our study the body max index is not significantly different in the population with postoperative atrial fibrillation compared to the population without postoperative arrhythmia.

Fat infiltration

The strong association found in this study between epicardial fat, but not with paracardial fat, and atrial fibrillation tends to confirm what was previously only hypothesised or observed in animal models. The findings suggest the pathophysiological role of epicardial fat in promoting the creation of an atrial arrhythmic substrate. Indeed, the different histological and functional nature of the two different types of epicardial and paracardial fat, and the close relationship of physical contiguity without anatomical barriers between epicardium and epicardial fat, allows the direct infiltration of the latter which can cause an alteration of atrial electrical properties.

In Friedman DJ et al.[26] a study was conducted on pericardial fat and the relationship with atrial conduction alterations on a surface ECG. An increased representation of pericardial fat as an independent predictor of P-wave index alterations in favour of altered atrial conduction was found. In addition, as previously demonstrated in the ovine animal model, epicardial fat could directly infiltrate the atrial myocardium causing microstructural alterations that are the basis of anatomical and electrical atrial remodelling that create the substrate for the development of re-entry arrhythmias.[18] From our research's available data, the difference in adipose infiltration of the atrial wall in the arrhythmic population compared to the non-arrhythmic population is not statistically significant, most likely due to insufficient sample numbers. An increase in the sample size may lead to different results revealing a higher adipose infiltration of the atrial wall.

There is little research in the literature on adipose infiltration of the atrial wall also because the imaging methods currently available do not allow to quantify the fat infiltrated in the thickness of the atrial wall due to its very thin structure. In this context more reliable data could be provided by a biopsy, which however cannot be performed *in vivo* outside of the group of patients who already have an indication for cardiac surgery. For further knowledge about the degree of fat

infiltration in patients developing atrial fibrillation, it is necessary to conduct further studies with a larger sample size, although the organisational complexity and costs of similar studies may make them difficult to implement.

The role of inflammation

Epicardial fat, in direct contact with the cardiac surface, has cardioprotective properties through biochemical, mechanical and thermoregulation functions and is a source of free fatty acids. These activities are also favoured by a dense network of vasa-vasorum. However, under pathological conditions this tissue may undergo changes in secretory properties with a pattern of pro-inflammatory cytokines, promoting a proatherogenic pathological condition and contributing to insulin resistance of the myocardium by secretion of TNF-alpha. This inhibits the insulin receptor and increases the release of non-esterified fatty acids. What regulates this balance is still unclear. Myocardial infiltration by fat in closer contact with the myocardium could trigger the cascade of inflammation and initiate the fibrotising process of the intercellular spaces, which is also the basis of the histological substrate changes observed in re-entry arrhythmias like atrial fibrillation.

Atrial fibrosis

The histological analysis of this study showed a statistically significant higher percentage of fibrosis in the group of patients who developed atrial fibrillation. This factor is also an arrhythmic event predictor independently from the other variables. The infiltration of epicardial fat under pathological conditions could be at the origin of the pathogenetic process that leads to intra myocardial fibrosis of the interstitial spaces and the arrhythmogenic substrate that is created. The epicardial fat in fact, and in particular its infiltration, could play a central pathogenetic role contributing to myocardial fibrosis of the adjacent myocardium. This may occur through paracrine activity with the secretion of profibrotic factors, such as pro-inflammatory cytokines, growth factors and metal proteases. In Werner S et al.[27] when the secretoma of interventricular and atrioventricular epicardial fat, obtained during surgery of sinus rhythm patients, was applied *in vitro* on the murine atrium, it induced massive myocardial fibrosis in a few days.

If the secretoma of the subcutaneous adipose tissue of the same patient was used in the same way, it had no effect on the atrial myocardium. These different results reflect the different nature of the two tissues, indicating the profibrotic effect as specific to epicardial fat, with the ability to transform fibroblasts into myofibroblasts. Intramyocardial fibrosis plays a central role in the de-structuring of the original atrial histological architecture through the creation of anatomical and functional barriers that lead to the formation of uni/bidirectional block points and lines of the electrical signal conduction. The substrate thus modified is also characterised by a shortening of the refractory periods, which facilitates the shortening of the wavelength and the reduction of the excitable gap. These modifications are at the basis of the onset of the perpetuation of complex re-entry arrhythmias such as atrial fibrillation. In the ovine model of comparison between obese and normal weight sheep, the electro-anatomical endocavitary study of the left atrium with Carto system (Biosense Webster) showed minor voltages in the posterior atrial wall with a greater heterogeneity of voltages in the group of obese sheep. In addition, this group of sheep, in the analysis of the histological preparation, had a significantly higher percentage of fibrosis than normal weight sheep.[18]

Diastolic dysfunction

In regard to the atrial parameters measured using echo method, the atrial dimension is significantly greater in patients who develop atrial fibrillation. It is also a predictor of arrhythmia independent of the other factors with an odds ratio of 1.05. Numerous studies confirm the importance of the atrial size as a predictor of atrial fibrillation. In a study with 3467 enlisted CT patients who underwent atrial size and pericardial fat measurement, atrial size was a strong independent predictor of atrial fibrillation [2.70 (2.22 - 2.20), P 0.0001], while epicardial fat was not.[28] Atrial size is usually the cause and/or consequence of atrial remodelling, which in turn involves electrical remodelling favouring the substrate for atrial fibrillation. In addition to this, other known predictors of atrial dysfunction such as E/A ratio were found to be related to the onset of postoperative atrial fibrillation. These elements are generally early indicators of underlying atrial

dysfunction and/or remodelling. In this context the atrial dimensions and the E/A ratio can be considered as a marker of a functional arrangement of an initial modification of the substrate in an arrhythmogenic sense, even before the arrhythmia has occurred.

CONCLUSION

Postoperative atrial fibrillation in patients undergoing aortic coronary artery bypass surgery is related to an increased presence of epicardial fat. From a histological analysis of the atrium in patients who develop postoperative atrial fibrillation there is an increased amount of fibrosis. From a pathophysiological point of view epicardial fat may play a fundamental role in the development of atrial fibrillation in two ways: direct infiltration causing an alteration of atrial structural and electrical properties and indirectly through paracrine activity of inflammation modulators and oxidative stress on the myocardium, which in turn promotes fibrosis. In this scenario the microscopic/histological alterations observed could be considered early markers of substrate evolution in an arrhythmic sense and confirm the pathogenetic importance for the development of atrial fibrillation. Lastly, age, atrial size and amount of atrial fibrosis are independent predictors of postoperative atrial fibrillation development.

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