GRIGORE T. POPA UNIVERSITY OF
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HABILITATION THESIS

RESEARCH IN CARDIOVASCULAR PATHOLOGY: FROM CLINICAL TO DIAGNOSTIC APPROACH

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Contents

Abbreviations

AA – aortic aneurysm
AAA – abdominal aortic aneurysm
ACR – American College of Rheumatology
AIA – anterior interventricular artery
AHA – American Heart Association
AHT – arterial hypertension
AF – atrial fibrillation
ARVC – Arrhythmogen Right Ventricle Cardimyopathy
AS – Angiosarcomas
ASR – Atrial structural remodeling
AT – adipose tissue
AT – atrophy
ATM – adipose tissue macrophages
ATS – atherosclerosis
AV – atrioventricular
BAV – bicuspid aortic valve
CA – cardiac adipocytes
CABG – coronary artery bypass
CAD – coronary artery disease
CEA – carotid endarterectomy
CCA – common carotid artery
ECA – external carotid artery
ICA – internal carotid artery
CBN – contraction band necrosis
CBP – cardiopulmonary bypass
CCA – common carotid artery
CD – cardiac sarcoidosis
CM – cardiomyocyte
CMP – cardiomyopathy
CRP – C reactive protein
CN – calcified nodule
CS – cardiac sarcoidosis
CT – cardiac tumors
CT – computed tomography
CTD – connective tissue disease
CVD – cardiovascular disease
CX – circumflex artery

DCM – dilated cardiomyopathy
DM – diabetes mellitus
ICA – internal carotid artery
IEL – internal elastic lamella
IHC – immunohistochemistry
IF – interstitial fibrosis
ITA – internal thoracic artery
ITI – intimal thickness index
EAT – epicardial adipose tissue
ECA – external carotid artery
ECM – extracellular matrix
EF – elastic fibers
EMB – endomyocardial biopsy
EVG – elastic Van Gieson
FA – fibro-atheroma
FC – fibro-calcified
FS – fibrosarcomas
GCA – giant cell arteritis
GHRL – ghrelin
GHS-R – growth hormone secretagogue receptor
HCM – hypertrophic cardiomyopathy
HE – hematoxylin-eosin
HL – hyperlipidemia
HPF – high power field
HT – hypertrophy
HTA – hypertension
LAD – left anterior descending artery
LAE – left atrial enlargement
LIMA – left internal mammary artery
LMS – leimyosarcomas
LVV – large vessel vasculitis
MAD – mean adipocyte diameter
MD – medial degeneration

- mMD – mild medial degeneration
- MMD – moderate medial degeneration
- SMD – severe medial degeneration

MF – medial fibrosis
MI – myocardial infarction
MRI – magnetic resonance imaging
MVCAD – multi-vessel coronary artery disease
PA – pericardial adiposity
PE – plaque erosion
POAF – perioperative atrial fibrillation

POSR – perioperative sinus rhythm
PR – plaque rupture
RA – radial artery
RAA – right atrial appendages
RCA – right coronary artery
RCM – restrictive cardiomyopathy
RCDD – rare cardiovascular diseases and disorders
RF – risk factor
RMS – rhabdomyosarcomas
SMC – smooth muscle cells
SP – stable plaque
SV – saphenous vein
SVG – saphenous vein grafts
TA – Takayasu arteritis
TAA – thoracic aortic aneurysm
TCFA – thin cap fibroatheroma
VP – vulnerable plaque
VSMC – vascular smooth muscle cells
USP – unstable plaque
WHO – World Health Organization
WPW – Wolff–Parkinson–White

Abstract

My habilitation thesis entitled "Research in cardiovascular pathology - from clinical findings to diagnostic approach" summarizes the main results of my scientific, professional and academic research, being generally focused on cardiovascular pathology. All my scientific work has been performed in the clinical and experimental pathology field and has been based on my expertise acquired during my doctoral training programme and enriched ever since.

The thesis includes three main sections divided, in their turn, in several chapters.

SECTION I, entitled "Professional, scientific and academic contributions", comprises three chapters, each devoted to a priority line of research for my professional portfolio. This section includes the most relevant scientific results obtained in my research, the presentation of which is connected to the existing information in the main publication flow.

Thus, each chapter relies on a short overview of the known and accepted literature data which motivated my research and also includes the presentation of the methods used and results achieved, followed by their interpretation, and critical and constructive analyses.

Chapter 1, entitled "Etiopathological profile of aortic aneurysm", has as its starting point the current data that are focused on the study of aortic aneurysms, and refers to risk factors, etiology and pathological aspects. Our study emphasized the main role of the histological examination of surgical biopsies to accurately establish the type and degree of injury in relation to the potential risk factors. The immunohistochemical study of inflammatory markers completed the diagnostic panel of a comparative study of two rare entities like the Giant Cell Aortitis and Takayasu Aortitis, both associated with aortic aneurysm.

The investigation topic targeted in **Chapter II** is postoperative atrial fibrillation, an increasingly common complication of heart surgery. Our study, conducted on right auricular biopsies, revealed the etiopathological profile of the postoperative fibrillating heart, comparing the clinical data and the results of the histopathological examination in patients with postoperative atrial fibrillation and in those with sinus rhythm, respectively. The actual contribution to the current state of knowledge consists of the full diagnostic monitoring and the identification of new risk factors, including inflammatory markers, proven by immunohistochemical means. Our research has thus identified, in patients with coronary artery diseases, myocardial cell differentiation lesions, which are adaptive reactions to various ischemic conditions. The immunohistochemical and morphometric study also revealed that myocardial and epicardial inflammation is expressed differently in various ischemic states, being more prominent in patients with postoperative atrial fibrillation compared to those remaining in sinus rhythm.

Chapter III contains 5 subchapters.

Subchapter 1, "Insights in the atherosclerotic disease", has as starting point the current data regarding the study of arterial pathology, respectively of ATS, with reference to the main risk factors involved in the etiopathogenesis, as well as the new research regarding the inclusion in this list of some morphological risk factors. This subchapter also presents the results obtained in a study meant to assess the pre-existing lesions in vessel grafts used in aorto-coronary bypass allowing the prediction of late graft-patency.

Subchapter 2, "Inflammatory myocardial diseases", is built on the basis of microscopic morphology evidence specific to myocardial inflammatory disease. Starting from the major role of myocardial endobiopsy, associated with immunohistochemistry techniques, as well as with imaging investigations in completing the diagnostic panel, the study presents the results obtained in the diagnosis of 2 rare entities, namely cardiac sarcoidosis and cardiac echinococcosis. Our diagnostic experience includes the evaluation of rare cases whose understanding has contributed to the current knowledge acquired in the field, as complementary data to be considered. From these rare diseases, cardiac sarcoidosis remains a challenge despite the progress in precocious myocardial disease diagnosis. As to primary echinococcus infection of the heart, this rare type of cystic echinococcosis, should be included in the differential diagnosis of cardiovascular disease in patients from endemic areas.

Sub-chapter 3, entitled "Primary cardiomyopathies", brings into disscussion the role of modern IHC techniques and genetics, and the value of their applicability in the diagnosis of cardiomyopathies. Arrhythmogenic cardiomyopathy is a rare but important cause of sudden cardiac death in the young. The chapter includes the presentation of our unique experience in arrhythmogenic right ventricular cardiomyopathy diagnosis.

Subchapter 4, "Myocardial infarction and Wolff – Parkinson – White syndrome" brings to our attention a rare association between unexpected acute ischemic heart disease and WPW syndrome, a rare condition, whose recognition is important not only in order to establish an appropriate therapy, but also to prevent its fatal evolution.
Our effective contribution to the current state of knowledge consists in the recognition and accurate diagnosis of these rare cases encountered in current clinical practice.

Sub-chapter 5, "Primary cardiac malignancies", brings to attention the high value of interdisciplinary collaboration in approaching rarely encountered cardiac tumors in clinical practice. These tumors are very rare, mostly representing case reports and experiences of single institutions. Therefore, individual approach to every case is extremely important and diagnosis and treatment options should be discussed thoroughly in multidisciplinary teams.

In what all these rare myocardial diseases are concerned, I have made numerous contributions in this field over the years, and they have all been materialized in books and book chapters, papers and communications.

SECTION II, entitled "**Scientific carrier and future research development**", is devoted to future projects that I would like to develop based on my prior research and the opening of new perspectives for further studies, in which I intend to attract new partners. An element of great interest is the in-depth study of inflammation related to the onset and evolution of atherosclerotic disease, whose development is also influenced by perivascular adipose tissue

(PVAT), which may be a determining factor in the persistent inflammatory state of the atherosclerotic plaque. Last but not least, the wide application of EBM is another major interest, not only as a useful diagnostic method, but also as a clinical research tool. Academic development plans include the drafting of teaching aids for students, the involvement of students, young doctors and Ph.D. students in pathological morphology research activities.

SECTION III includes the list of references, which supports the information included in this habilitation thesis.

Rezumat

Teza de abilitare intitulată ”Cercetări în patologia cardiovasculară - de la clinică la abordare diagnostică” prezintă rezultatele principale ale activității mele științifice, profesionale și academice, fiind centrată în general pe domeniul patologiei cardiovasculare. Toate lucrările mele științifice au fost realizate în domeniul patologiei clinice și experimentale pe baza experienței dobândite în timpul programului de formare doctorală și continuată ulterior.

Teza este structurată în trei secțiuni principale, subdivizate la rândul lor în capitole.

SECȚIUNEA I, intitulată „Contribuții profesionale, științifice și academice, este organizată în cinci capitole, fiecare dedicat unei direcții de cercetare prioritare pentru portofoliul meu profesional. Această secțiune include cele mai relevante rezultate științifice obținute în cercetare, a căror prezentare este conectată la informațiile existente în fluxul principalelor publicații. Astfel, fiecare capitol este susținut de o scurtă privire de ansamblu asupra datelor cunoscute și acceptate din literatura de specialitate care a motivat studiile și include ulterior prezentarea metodologiei utilizate și a rezultatelor personale obținute, urmate de interpretare, discuții critice constructive și concluzii.

Capitolul 1, intitulat “Profilul etiopatologic al anevrismului aortic” are ca punct de plecare datele actuale care se concentrează pe studiul anevrismelor aortice, referindu-se la factori de risc, etiologie și aspecte patologice. Studiul nostru s-a bazat pe rolul principal al examenului histologic pe biopsii chirurgicale pentru stabilirea cu acuratețe a tipului și gradului lezional în raport cu factorii de risc potențiali. Studiul imunohistochimic al markerilor inflamatori a completat panoul de diagnostic, în studiul comparativ a două entități rare, și anume aortita cu celule gigante și aortita Takayasu, ambele asociate anevrismului aortic.

Capitolul II are ca subiect de cercetare fibrilația atrială postoperatorie, o complicație tot mai frecventă după intervenția pe cord. Studiul nostru, efectuat pe biopsii auriculare drepte, a relevat profilul etiopatologic al inimii fibrilante postoperator, analizând comparativ datele clinice și rezultatele examenului histopatologic la pacienții cu fibrilație atrială postoperatorie și respectiv la cei rămași în ritm sinusal. Contribuția personală reală la starea actuală a cunoștințelor constă în monitorizarea completă a diagnosticului și identificarea unor factori de risc noi, ce includ markeri inflamatori, demonstrați imunohistochimic. Cercetarea a identificat astfel, la bolnavii coronarieni, leziuni de diferențiere a celulelor miocardice, care reprezintă reacții de adaptare la condiții ischemice diverse. Studiul imunohistochimic și morfometric a relevat, de asemenea, că inflamația miocardică și epicardică sunt exprimate diferit în stări ischemice variate, având un grad mai mare la pacienții cu fibrilație atrială postoperatorie comparativ cu cei rămași în ritm sinusal.

Capitolul III conține 5 sub-capitole.

Subcapitolului 1, “Informații despre boala aterosclerotică”, are ca punct de plecare datele actuale ce vizează studiul patologiei arteriale, respectiv a ATS, cu referire atât la

principalii factori de risc implicați în etiopatogenie, cât și la noile cercetări privind includerea în această listă a unor factori de risc morfologici. În acest subcapitol sunt prezentate și rezultatele obținute în cadrul unui studiu de evaluare a evoluției leziunilor ATS efectuat pe vase utilizate în bypassul aortocoronarian, care pot prezice permeabilitatea la distanță a grefei vasculare.

Subcapitolul 2, "Boli inflamatorii miocardice", este construit în baza evidenţelor de morfologie microscopică specifice bolii inflamatorii miocardice. Pornind de la rolul major al endobiopsiei miocardice, al tehnicilor de imunohistochimie, precum și a investigatiilor imagistice în completarea paletei diagnostice, studiul prezintă rezultatele obţinute în diagnosticul a două entități rare, sarcoidoza şi echinococoza cardiacă. Experiența noastră de diagnostic include evaluarea cazurilor rare a căror înțelegere a contribuit la cunoștințele actuale dobândite în domeniu, furnizând detalii suplimentare. Din aceste boli rare, sarcoidoza cardiacă rămâne o provocare în ciuda progreselor în diagnosticul precoce al bolii miocardice. În ceea ce privește infecția primară cardiacă cu echinococus, acest tip rar de echinococcoză chistică trebuie inclus în diagnosticul diferențial al bolilor cardiovasculare la pacienții din zone endemice.

Subcapitolul 3, intitulat *"*Cardiomiopatii primare", aduce în prim plan rolul tehnicilor moderne de IHC și genetică, și valoarea aplicabilității lor în cercetarea cardiomiopatiilor. Cardiomiopatia aritmogenă este o cauză rară, dar importantă de moarte subită cardiacă la tineri. Capitolul include experiența noastră unică in diagnosticul cardiomiopatiei aritmogene ventriculare drepte.

Subcapitolul 4, "Infarctul de miocard și sindromul Wolff – Parkinson – White" supune atenției o asociere rară între ischemia miocardică acută neașteptată și sindromul WPW, afecțiune rară, a cărei recunoaștere este importantă nu numai în vederea instituirii unei terapii adecvate, dar și pentru prevenirea evoluției sale fatale. Contribuţia noastră efectivă la stadiul actual al cunoaşterii constă în recunoasterea și diagnosticul de acuratețe al unor cazuri cu patologie rar întâlnită în practica clinica curentă.

Subcapitolul 5, "Malignități cardiace primare", aduce în prim plan preocuparea majoră pe care am avut-o de realizare a unei colaborări interdisciplinare în abordarea unor tumori cardiace rar întâlnite în practica clinică. Aceste tumori cardiace sunt rare, reprezentând în mare parte rapoarte de caz și experiențe singulare ale instituțiilor. Prin urmare, abordarea individuală a fiecărui caz este esențială, iar diagnosticul și opțiunile de tratament ar trebui discutate în detaliu în echipe multidisciplinare.

În ceea ce privește toate aceste boli miocardice rare descrise, am adus numeroase contribuții în acest domeniu de-a lungul anilor și toate s-au concretizat în cărți și capitole de carte, lucrări și comunicări.

SECȚIUNEA a II-a, intitulată „Planuri de dezvoltare a carierei și evoluția viitoare a cercetării", este dedicată proiectelor viitoare pe care aș dori să le dezvolt pe baza cercetărilor mele anterioare, precum și deschiderii unor perspective noi pentru studii ulterioare, în care

intenționez să atrag noi colaboratori. Un element de mare interes este studiul aprofundat al inflamației legate de debutul și evoluția bolii aterosclerotice, a cărei dezvoltare este influențată și de țesutul adipos perivascular (PVAT), care poate fi un factor determinant în starea inflamatorie persistentă a plăcii aterosclerotice. Nu în ultimul rând, aplicarea pe scară largă a EBM prezintă un interes major, nu numai ca metodă utilă de diagnostic, ci și ca instrument de cercetare clinică. Planurile de dezvoltare academică includ dezvoltarea materialelor didactice pentru studenți, implicarea studenților, a tinerilor medici și a doctoranzilor în activitățile de cercetare morfopatologică.

SECȚIUNEA III include lista de referințe, suportul informațiilor existente în această teză de abilitare.

Introduction

I have a professional experience that covers 21 years of hospital practice and 30 years of academic activity - beginning in September 1991, when I have won my first academic position of University Assistant by competitive examination at the Morphopathology Discipline at the "Grigore T. Popa" University, Iasi.

I graduated from the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi in 1982 with the highest grade, and then I was trained for 3 years as a probationer of the Public Health Hospital in Iasi, as were the rules at that time. It was a very good medical experience as I worked in 6 different specialties, which prepared me for my next step - General Practitioner at the Medical Dispensary of Ciortesti, Iasi District, Romania, where I worked for 2 years. In 1987, I took the national residency examination and I was admitted as resident in Epidemiology and Infectious Diseases and in July 1991 I became a specialist in the same specialty.

The onset of my professional career started in 1991, when I participated in the competitive examination for the position of University Assistant at the Morphopathology Discipline, Faculty of Medicine, Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Romania. The following year I was admitted as resident in Pathological Anatomy and for doctoral degree (PhD) studies to Professor Gheorghe Scripcaru. From this period on my professional life evolved as a mixture between professional and academic training. In 1994, I became junior specialist in Pathological Anatomy.

In 1998, I was awarded the PhD deghree – i.e. a Doctor of Medical Sciences - with a thesis focusing on pediatric pulmonary pathology as a cause of death. For my doctoral thesis I had a good cooperation with the Electron Microscopy Laboratory of the Cell Biology Department of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi. The theme of my PhD thesis was the starting point for a book centered on Sudden Infant Death Syndrome – SIDS.

I was acknowledged Senior Specialist in Pathological Anatomy in 1998, and in the same year I was promoted to Assistant Professor in Morphopathology, continuing the symbiosis between professional and academic didactic activity.

From 2000, I work at the "Prof.dr. GIM Georgescu" Cardiovascular Disease Institute of Iasi, in the Anatomical Pathology Laboratory. The last 20 years of activity in this field were in equal measure years of professional, didactic and scientific activity.

In 2003, I became Associate Professor of Pathology at the "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

The experience in cardiovascular pathology was also achived during my Cardiovascular Training in Freiburg in Albert-Ludwig's University and London at Royal Brompton Hospital and Harefield NHS Foundation Trust. The most prominent events during my postdoctoral evolution were the collaborations with prestigious groups of cardiopathologists from Romania (Prof. I. Florescu, MD, PhD - Cluj-Napoca), from The Netherlands (Prof. Anthon Becker, MD, PhD) and England (Margaret Burke, MD, PhD), resulting in significant scientific publications

in ISI Journal of papers, mainly case reports, presenting and debating on rare cardiovascular conditions.

My academic career developed at the same time with my professional achievements. Throughout the years, this helped me to establish a connection based on respect and mutual trust with students, residents and young researchers. As a result of this fruitful cooperation, I have always been involved in student manifestations, as coordinator for those interested in research, including student congresses and final project preparation.

My teaching interest focused on the post-academic segment, being coordinator and lecturer for several courses of continuing medical education (CME) dealing with different specialties.

The interest in teaching the young generation materialized in the publication of numerous courses, practical works manuals, and medical books, as unique author.

In 2005, I published a Pathology Course destined for Romanian language section students of the Medicine Faculty, in 2010 appeared the English version of the course, and in 2017 I wrote a second edition, with more information in this rapidly developing domain. In 2012 I published separate sets of course books for English language section students of the Medicine Faculty and Dental Medicine Faculty.

I am the unique author of three "Practical works of Pathological Anatomy", one for Romanian language section students of the Medicine Faculty, and two for English language section students of the Medicine Faculty and Dental Medicine Faculty.

I am co-author of another teaching material: "Practical Works of Pathological Anatomy", under supervision of Prof. Dr. Maria- Sultana Mihailovici (1999).

Another important part of my teaching activity is the constant preoccupation in residency program. I am the coordinator of the residency program in Cardiovascular Pathology. I am working with great passion and interest when I am preparing these courses and practical activities, whose content depends on the group of medical specialties I address.

In 2011, I published the book "Morpho-clinical Confrontations" and in 2015, an Atlas of Cardiovascular Pathology, both books entirely dedicated to students and residents in Pathology and other medical specialties.

As a teacher, I participated objectively in numerous examination and contest commissions and I trained and encouraged dozens of generations of students, some of whom became specialists in the same field, trying to cultivate in them the love for pathological anatomy.

In teaching, I always tried to introduce the latest advances and updates of contemporary medicine, correlating the theoretical notions of pathology with clinical and experimental pathology.

At academic level, I was involved in various activities of the university community, of which I mention: (a) member in commissions for teaching staff promotion at "Grigore T. Popa" University of Medicine and Pharmacy Iasi (2000 - present); (b) member of the doctoral thesis supervision committees (2014 - present).

In the field of research, I started with basic problems under the guidance of Prof. Lorica Gavrilita, MD, PhD, and then I passed through both experimental and morphoclinical studies, as well as applied anatomo-clinical problems.

As fundamental investigations, for instance, I took part in an investigation aimed at defining the involvement of the Aluminum from drinking water in the development of liver, kidney and brain damages (2002), and at describing the lesion related to chronic intoxication with Aluminum. The results appeared in papers published in 2002 in the Medical Surgical Journal of the Society of Physicians and Naturalists of Iasi.

My contribution to better understanding of thymus pathology was brought by writing a monograph and articles representing personal studies of histopathology, immunohistochemistry and morphometry, performed especially on tumors of the thymus.

I was member of the organizing committee of the cytology tutorials concluded with numerous IDB (International Data Base) papers referring to cytological diagnosis on pleural-pericardial effusions of the serosa primary and secondary tumors.

I was particularly interested, as main direction of my pathology research, in cardiovascular pathology. The results were published in both BDI and ISI medical journals: The Medical-Surgical Journal of the Society of Physicians and Naturalists of Iasi, Archives of Pathology, Surgical Journals, etc.

All the research activities were closely related to the practical activity aimed at diagnosing various cardiovascular conditions. I presented my results as communications in numerous scientific events, such as: national and international congresses or symposiums devoted to pathology. In 2010, I wrote a book on Cardiovascular Pathology News, summing up a decade's experience in this field.

One of my most important future goals is to broaden my research areas by building interdisciplinary teams with different other disciplines. I am already walking this path by building a team with Surgery and Physiopathology Disciplines.

International visibility is reflected by Web of Science H-index: 7; total number of citations: 66 (one paper has 17 citations); 20 books (18 as first author); 23 book chapters (16 as first author); 25 ISI papers (18 as main author, of which 5 as first author; 7 co-author); 46 IDB works (30 as first author; 16-coauthor); 18 Works in other journals or volumes of conferences with ISBN or ISSN (of which 7 as first author); activity in National grants: partner director (1 project), and team member: 3.

SECTION I. PROFESSIONAL, SCIENTIFIC AND ACADEMIC CONTRIBUTION

CHAPTER I. AORTIC ANEURYSM: FROM ETIOLOGY TO DIAGNOSIS

I.1. State of the art

The aorta, the largest artery in the human body, is divided into two large segments: the thoracic aorta and abdominal aorta (Davies et al, 1998). Aortic aneurysm (AA) represents a pathological dilatation of a segment of the aorta, with tendency to expansion and rupture. It can affect all segments of the aorta. By consensus, it is referred to aneurysm when the diameter of the dilated segment is 50% larger than normal for the respective area (for the abdominal aorta > 3 cm and for the ascending aorta > 4.5 cm). The dimensions of the aorta differ according to age, sex and body surface area. The ascending aorta and the arch of the aorta are about 3 cm in diameter. The descending thoracic aorta is between 2-2.3 cm and the abdominal aorta measures approximately 1.7-1.9 cm in the infrarenal segment. Aging leads to a slight and generalized dilation of the aorta (ectasia of the aorta). It is considered that the maximum diameter of the ascending aorta should not exceed 4 cm (Pomerance et al, 1997).

The pathobiology of aortic aneurysm (AA) is both complex and multifactorial, and is associated with several significant developmental risk factors. Understanding current concepts in the etiology and pathogenesis of AA is therefore imperative in future research studies and in aiding the development of treatment guidelines.

1. Aortic aneurysm pathogenesis

In 2001, the pathogenic mechanism of the abdominal aortic aneurysm (AAA) was summarised into four broad areas: proteolytic degradation of the aortic wall connective tissue, inflammation and immune response, molecular genetics and biomechanical wall stress (Wassef et al, 2001). More recently, other authors have investigated three possible patterns of AAA pathogenesis: (a) AAAs secondary to a local disease process confined to the atherosclerotic abdominal aorta, (b) genetic susceptibility and biochemical wall stress triggering systemic dilation of the aorta, and (c) diseased vascular tree as a consequence of a chronic inflammatory process (Nordon et al, 2011). They concluded that evidences suggest AAA disease as a systemic disease of the vasculature, with a predetermined genetic susceptibility, leading to a phenotype governed by environmental factors. AAAs are therefore referred by some researchers as a multifactorial degenerative disease (Michel et al, 2011; Nordon et al, 2011).

The major genetic factor in the appearance of aortic aneurysms could be an inborn defect of collagen type III or other components of the connective tissue matrix. At least 20% of aneurysms result from inherited disorders (Kuivaniemi et al, 2008).

AAAs are associated with atherosclerosis, transmural degenerative processes, neovascularization, degeneration of vascular smooth muscle cells, and a chronic inflammation, mainly located in the outer aortic wall (Hellenthal et al, 2009). Observational evidence now suggests that the intraluminal thrombus (ILT), together with adventitial angiogenesis and immune responses, play important roles in the evolution of atherothrombosis from the initial

stages through to clinical complications, which include the formation of aneurysms (Michel et al, 2011).

A number of other factors have also been commonly associated with aneurysm formation. They include family history, advanced age, male sex, hypertension, aortic dissection and arteriosclerosis (Sweeting et al, 2012).

2. Structural considerations in AA

Multiple factors rather than a single process are implicated in AA pathogenesis. These result in the destructive changes in the connective tissue of the media and adventitia of the aortic wall and ultimately lead to aneurysm formation and eventual rupture. The media is composed of multiple elastic laminae alternating with circularly oriented vascular smooth muscle cells (VSMCs) and surrounded by ground substance. The adventitia lacks lamellar architecture but is composed of loose connective tissue with fibroblasts and associated collagen fibers and vasa vasorum. Integrity of the aortic wall is dependent on balanced remodelling of the extracellular matrix (ECM), predominantly of elastin, collagen and VSMCs (Dobrin & Mrkvicka, 1994).

2.1. Elastin

The chief component of the media is elastin, a lamellar ECM protein consisting of soluble tropoelastin monomers. Elastin production by the VSMCs ceases when a patient reaches maturity, having a half life of 40 to 70 yrs (Dobrin et al, 1999).This could explain the elderly predisposition to AA formation.

Elastin is responsible for the load bearing property that behaves uniformly in both the circumferential and longitudinal directions at different locations across the wall thickness (Dobrin et al, 1999), thereby absorbing oscillating arterial shock waves, providing recoil and maintaining arterial structure.

2.2. Collagen

Collagen is the primary structural component of the arterial adventitia and has been identified in smaller quantities in the media, and is responsible for tensile strength and resistance of the arterial wall. In contrast to elastin, collagen is synthesized on a continual basis throughout life, thereby collagen content represents the net effect of synthesis and degradation. Type 1 fibrillar collagen accounts for aortic wall load bearing capability (over 20 times greater than that of elastin), while Type 3 collagen provides some extensile stretch (Pichamuthu et al, 2013). Arterial distension in response to increasing intraluminal pressures are limited through the recruitment of inextensible collagen fibers. Structural damage occurs when collagen is extended beyond 2–4% from its uncoiled form (Dobrin, 1999).

2.3. Vascular smooth muscle cells (VSMCS)

VSMCs as part of the ECM form an important structural element and perform a mediator role in AA disease by producing TGF-beta1, ECM and inhibitors of proteolysis (O'Callaghan & Williams, 2000). Transition of VSMCs from a contractile to a synthetic phenotype is characterized by a change in cell morphology, resulting in the production of substances such as components of the ECM, growth factors, and proteases, which are important in remodeling the vascular wall (Sakalihasan et al, 2005).

VSMC density depends on patient age, patient gender and the location of quantification in non-atherosclerotic aneurysms. Conversely, loss of VSMCs is a characteristic of

atherosclerotic aortic aneurysms (Kirsch et al, 2006). In particular VSMC apoptosis has been associated with fibrous cap thinning, enlargement of the necrotic core, plaque calcification, medial expansion and degeneration, elastin breaks, and failure of outward remodeling. In addition, chronic VSMC apoptosis may mimic multiple features of medial degeneration seen in a variety of human pathologies (Sakalihasan et al, 2005).

2.4. Structural considerations in TAA vs AAA

Elastin lamellar units are found less frequently in AAA as compared to TAA, with an even more marked difference infrarenally. This relative paucity of elastin and collagen is thought to play a role, amongst other factors, in the predisposition for aneurysm development in the infrarenal aorta.

The microscopic findings in TAAs are predominantly described as cystic medial degeneration, reflecting a non-inflammatory loss of medial VSMCs, fragmentation of elastic lamellae, and mucoid degeneration.

In contrast, the histopathologic features of AAAs are characterized by severe intimal atherosclerosis, chronic transmural inflammation, neovascularization, and destructive remodeling of the elastic media (Diehm et al, 2007).

Furthermore, ascending TAAs are associated with an underlying bicuspid aortic valve (BAV) with an estimated 75 % of patients who underwent BAV replacement demonstrating cystic medial necrosis on aortic biopsy. Inadequate levels of fibrillin-1 may be responsible for this weakness in aortic wall leading to BAV (Schmid et al, 2003).

3. Molecular genetics in AA

Aortic aneurysms are a complex multi-factorial disease with genetic and environmental risk factors. As already mentioned, genetic factors have been shown to play a role in the etiology of both TAA and AAA. The genetic basis of aortic aneurysms was reviewed in 2008 (Kuivaniemi et al, 2008).

The major determining factor in the appearance of aortic aneurysms may be an inborn defect of collagen type III or of another component of the connective tissue matrix. At least 20% of aneurysms result from inherited disorders (Kuivaniemi et al, 2008). Medial necrosis of the proximal aorta in aneurysms is associated with a number of conditions, including inherited connective tissue disorders such as Marfan syndrome and Ehlers—Danlos syndrome type IV. It can also be present along with bicuspid aortic valve, coarctation of the aorta, etc. (Caglayan & Dundar, 2009).

3.1. Genetic considerations in AAA

AAAs develop in 20% of brothers of patients with this condition (first-degree relative with an AAA) (Wahlgren et al, 2010). These include findings such as the presence of multiple aneurysms and systemic abnormalities in aneurysm patients, increased connective tissue laxity, all emphasizing a role for genetic factors in AAAs.

Genome-wide scans of these patients have suggested a role for genes located on chromosome 19q13 and 4q31.47. Candidate genes in these regions include interleukin (IL)-15, endothelin receptor A, programmed cell death, and LDL receptor-related protein (Kuivaniemi et al, 2015).

3.2. Genetic considerations in TAA

Once one aneurysm has been accidentally discovered, the patient is at increased risk for developing another aneurysm (Hasham et al, 2003). If any mutation is found in the affected patients, the mutation should then be equally investigated in their relatives, and hence genetic counseling should be given immediately after. Because of this increased risk related to target diseases, chromosomal and gene analyses are essential in selected cases with aneurysms in inherited forms (Caglayan & Dundar, 2009).

In pedigrees with several generations of multiply affected family members, chromosomal loci have been identified. These loci have been mapped to the 5q13-14, 11q 23.2-24, and 3p24-25 chromosome sites (Hasham et al, 2003). Most recently, important work has localized the mutation on the 3p24-25 chromosome to the transforming growth factor-receptor type II (Pannu et al, 2005).

Albornoz and his colleagues found that 79.5% TAA had an inheritance pattern that was most consistent with a dominant mode of inheritance, while 20.5% TAA were most consistent with a recessive inheritance pattern (Albornoz et al, 2006).

4. Conclusions

Interaction of multiple factors rather than a single process is responsible for the failure of the integrity of the aortic wall, which result in AA formation and progression.
Despite several similarities in etiology and pathogenic mechanisms, it appears that TAA differs in many ways from AAA.

Current areas of interest include proteolytic degradation of the arterial wall, inflammation and the immune response, biomechanical wall stress, and molecular genetics.
Knowledge of the pathobiology of AA has lead to more targeted imaging methods and treatment trial design to investigate various pathobiological mechanisms of AA progression.

Future research should take into consideration knowledge gained of the differences between TAA and AAA pathobiology, in order to unravel the specificities of these different events in AA.

The interest in this field lead to results published in 9 scientific papers (6 in journals indexed in international databases and 3 in journals indexed in Clarivate Analytics)(former ISI database). The papers published in journals with impact factor (Clarivate Analytics) are presented below:

1. **Butcovan D**, Mocanu V, Baran D, Ciurescu D, Tinica G. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens. *Exp Ther Med* 2016; 11(5): 2028-2032. **IF-1.261**
2. **Butcovan D**, Mocanu V, Haliga RE, Ioan BG, Danciu M, Tinică G. Sub-classification of non-inflammatory and inflammatory surgical aortic aneurysms and the association of histological characteristics with potential risk factors. *Exp Ther Med* 2019; 18 (4): 3046-3052. **IF=1.448**
3. **Butcovan D**, Tinica G, Statescu C, Timofte D, Ciuntu B, Sascau R, Radu R, Anghel L, Schaas MC, Badescu C, Lupusoru RV. Immunohistochemical Interplay in associated Large Vessel Vasculitis and consequent Ascending Aortic Aneurysm and Review of Literature. *Rev Chim* 2019; 70 (12): 4348-4353. **IF=1.605**

I.2. Etiopathological characteristics of the aortic aneurysms correlated to clinical data and aortic surgical biopsy results

I.2.1. Introduction

An aortic aneurysm is an abnormal enlargement of the aortic wall. Etiologically, the common risk factors of AA, whatever the aortic segment is affected, are: smoking, age, male sex, high blood pressure, hypercholesterolemia, family history. This suggests a genetic predisposition. There are certain diseases with hereditary transmission in which the aortic (usually ascending aorta) aneurysm appears: Marfan syndrome, Ehlers-Danlos vascular syndrome, Loyes-Dietz syndrome, bicuspid aortic valve (Stary et al, 1995). The aorta wall has three layers: the inner layer or intima, the middle layer or media and the outer layer called adventitia. In AA, these layers show various lesions, located in one or more of the mentioned layers, in relation to the associated aortic disease. There is a large number of aortic diseases, these being part of the broad pathological categories in which the degenerative and inflammatory changes interact in a complex manner. Today, the histopathological examination of the surgical samples is an essential component of the diagnosis of the aortic disease (Halushka et al, 2016). Despite the increasing clinical-therapeutic complexity of the aortic pathology, the histopathological diagnostic criteria have not been adequately reassessed over the years, leading to discrepant terminology and poor standardization of diagnostic criteria (Stone et al, 2015). The AHA members have proposed a consensus document, which aims to define the characteristics of the histopathological substrate in the main non-neoplastic aortopathies (more precisely non-inflammatory and inflammatory aortic pathology) and to systematize the diagnostic criteria for these pathologies of the aorta (Leone et al, 2012)

Diagnosis in aortic pathology requires more attention and little is known about the histological patterns of aortic pathology with regard to medial degeneration, atherosclerosis and aortitis as related to potential risk factors as well as to their distribution in different aortic segments. While medial degeneration is reported to be the leading histological finding in cases of aneurysm, the role of atherosclerosis and inflammatory processes seems to be underestimated (Halushka et al, 2016; Stone et al, 2015). A form of clinical manifestation of all these aortic lesions is aortic aneurysm. As a permanent dilatation of the aortic wall, aortic aneurysm may interest all aortic segments, thoracic aorta and abdominal aorta (Leone et al, 2012).

Objective

Our study aimed to evaluate etiopathology of the aortic aneurysm including medial degeneration (MD), atherosclerosis (ATS) and inflammatory processes (aortitis) on aortic surgical biopsies in relation to potential risk factors.

I.2.2. Material and methods

Fifty two intraoperatively obtained specimens of the entire aorta were analyzed. The specimens included 39 thoracic and 13 abdominal aortic aneurysms from 38 men (73%) and 14 women (27%) with age ranged from 19 to 80 years (average, 61.7 + 15 years). Patients were stratified according to the aortic segments involved.

Information on individual clinical data (age, sex hypertension, smoking, history of the aortic disease, and previous inflammatory process) were obtained from medical records.

Histological analysis of the aortic specimens was performed. Hematoxylin-eosin staining was used routinely as a standard diagnostic tool. Elastic Van Gieson's staining was used to analyze elastic and collagen fibers and alcian blue staining was used to detect acid mucopolysaccharides. Movat pentachrome stain was used for all wall structure components including mucoid material. Magnifications of 40×, 100×, 200× and 400× were used. Histological assessment was performed by using an optical microscope (CX41; Olympus Corporation, Tokyo, Japan). The measurements were visualized using color image analysis software (Quick Photo Micro 3.0.).

For optimizing the aortic histological diagnosis we applied the standardized nomenclature in keeping with consensus documentation referring to unified nomenclature for a variety of both aortic noninflammatory degenerative lesions and inflammatory ones (Leone et al., 2012). ATS lesions were found in both TAA and AAA cases, presenting various degrees of severity with consequent aortic wall changes. The severity of medial degenerative and atherosclerotic lesions in surgically resected segments of aorta (mild-1; moderate-2; severe-3) was graded according to ESC guidelines (Stone et al., 2015).

Statistically, we analysed the association of potential aortic aneurysm risk factors with the histological characteristics of thoracic and abdominal aortic aneurysms: medial degeneration (MD), atherosclerosis (ATS) and aortitis. Data are expressed as percentages, calculated using Excel software (Microsoft Corporation, Redmond, WA, USA).

I.2.3. Results

An aneurysm is defined as a dilatation of at least 50% above the normal diameter of an artery. Out of the total number of 52 patients suffering from aortic dilation (aortic aneurysm) that we investigated, the thoracic aorta resulted to be the segment most frequently involved (n=39), while the abdominal aorta was affected in only one third of cases (n=13). Aneurysms in the two aortic segments we considered in this study had different etiologies.

In fact, as previouslşy outlined, aortic aneurysms have a multifactorial etiology. The multiple risk factors included advanced age, male gender, smoking, arterial hypertension (AHT), atherosclerosis (ATS), and bicuspid aortic valve (BAV) lesions.

Patients' average age in TAA was 57.2 years in males and 49.2 years in females, whereas in AAA it was 68.1 years in males and 72.2 years in females.

From the entire aortic aneurysm group (n=52), male gender predominanted in both thoracic aortic aneurysm (TAA) group (79.4%, 31 out of 39 patients) and abdominal aortic aneurysm (AAA) group (61.5%, 8 out of 13 patients).

When the risk factors were analysed, smoking was a commoner finding in TAA (58.9%; n=23) than in AAA (46.1%; n=6) groups. Similarly, HTA registered higher values in TAA (33.3%; n=13) than in AAA (23%; n=3) patients. BAV injury was found only in four TAA cases (17.9%) in association with aortic regurgitation. We did not find any genetic disorder history in the study group patients.

Histopathological examination revealed medial degeneration (MD) lesions (64.1%, 25 of 39 patients), mixed lesions, MD and ATS (17.9%, 7 of 39 patients), along with microscopically proven aortitis (12.8%, 5 of 39 patients) in TAA patients (n=39), while in AAA patients (n=13) only ATS lesions (100%) were present.

Medial degenerative lesions in aortic aneurysm

We described various cases of medial degeneration (MD) leading to TAA in 25 patients. Of all 25 MD aortic lesions, 3 cases (12%) displayed mild medial degeneration (mMD), 10 (40%) moderate medial degeneration (MMD) and 12 (48%) severe medial degeneration (SMD) (fig. I.2.1).

In the three TAA with mMD aortic lesions (grade 1), aortic medial wall showed multifocal decrease in the number of smooth muscle cells (SMCs) with a minimal collagen deposition, decrease in number of the elastic fibers (EF) and mild multifocal EF fragmentation associated with mild focal intralamellar mucoid accumulation (fig. I.2.1.A).

Other ten TAA cases presented a MMD substrate (grade 2). The aortic wall had a decreased number of SMCs and mild multifocal substitutive fibrosis, a reduction in number of EFs and moderate multifocal EF fragmentation with formation of moderate multifocal and focal both intralamellar and translamellar mucoid accumulation (fig. I.2.1.B).

In the last 12 TAA cases with SMD morphological substrate (grade 3) we found medial aortic wall showing extensive, band-like SMC loss, moderate multifocal EF fragmentation and severe multifocal translamellar mucoid accumulation (fig. I.2.1.C).

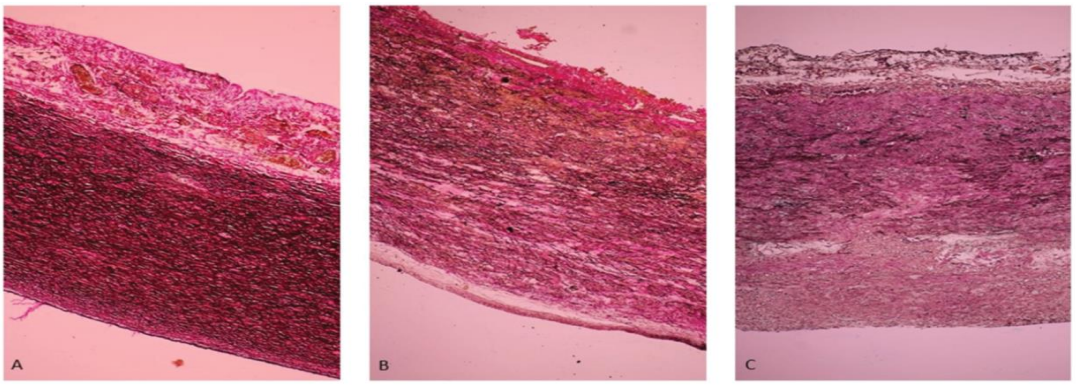

Figure I.2.1. Aortic medial degeneration. (A) Grade 1; (B) Grade 2; (C) Grade 3. Elastic Van Gieson's staining, x100.

Atherosclerotic lesions in aortic aneurysm

Of the 20 atherosclerotic aneurysms, 13 (65%) were abdominal and 7 (35%) were thoracic aneurysms. Patient groups included 15 males (75%) and 5 females (25%), with ages between 44 and 85 years.

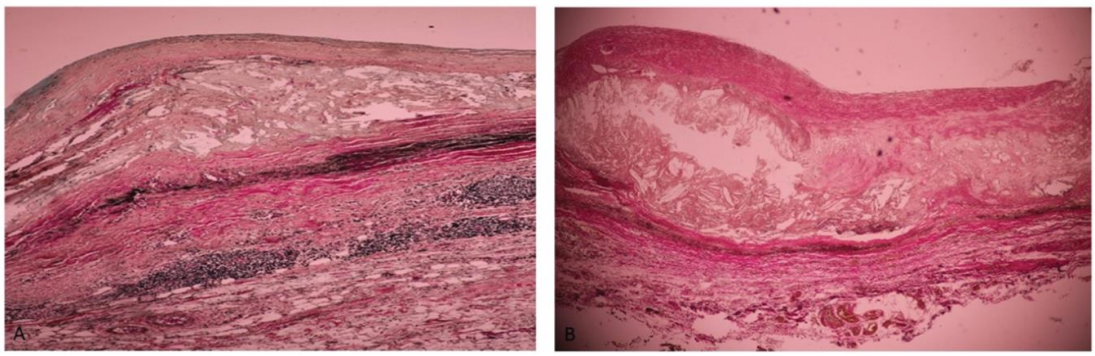

Figure I.2.2. (A) Mixed thoracic aortic aneurysm; (B) Atherosclerotic abdominal aortic aneurysm. (Elastic Van Gieson's staining, x100).

In TAA aneurysms, most cases had moderate to severe medial degeneration (MD) associated with intimal atherosclerotic lesions of different types (1-2 grades; grade 1 = intimal extracellular lipid deposition without fibrosis, AHA grade III/IV; grade 2 = extracellular lipid deposition with fibrosis involving less than 1/3 of the media thickness = AHA grade V), with formation of mixed, degenerative-atherosclerotic aneurysms (fig. I.2.2.A). In AAA aneurysms, all atherosclerotic lesions were severe (3 grade), with extracellular lipid deposition and fibrotic changes of the entire wall (AHA grade V), with or without associated complications (fig. I.2.2.B). In most AAA cases, we also found adventitial inflammation.

Our study revealed early atherosclerotic lesions in thoracic aorta (fig. I.2.2.A) and advanced atherosclerotic lesions in abdominal aorta (fig. I.2.2.B).

Inflammation in aortic aneurysm

Among TAA patients, five had aortitis proven by histological examination, including 3 GCA cases, one TA case and one patient with syphilitic aortitis.

The aortitis group included 3 men and 2 women (ratio of 3:2). The three giant cell aortitis (GCA) cases consisted of 2 males and 1 female (ratio of 2:1) with an average age of 62.5 years (range: 52 to 70); the Takayashu aortitis (TA) patient was a female of 36 years and the remaining patient suffering from syphilis complications was a male of 56 years.

In all five TAA aortitis cases, the mean preoperative erythrocyte sedimentation rate was 31.5 mm/h, and the mean preoperative value for C-reactive protein was 21.6 mg/dl, respectively. Of the 5 patients, 1 (20%) needed coronary artery bypass grafting, 3 (60%) underwent aortic valve surgery (1 aortic valve repair and 2 aortic valve replacement), and all 5 (100%) required aortic surgery (ascending aortic replacement).

In most aortitis cases (60%) the diagnosis was made after the operation, on the basis of histopathologic examination, except for the TA case where preoperatory imagistic examination suggested TA disease, and for the syphilitic aortitis in the patient known with syphilis.

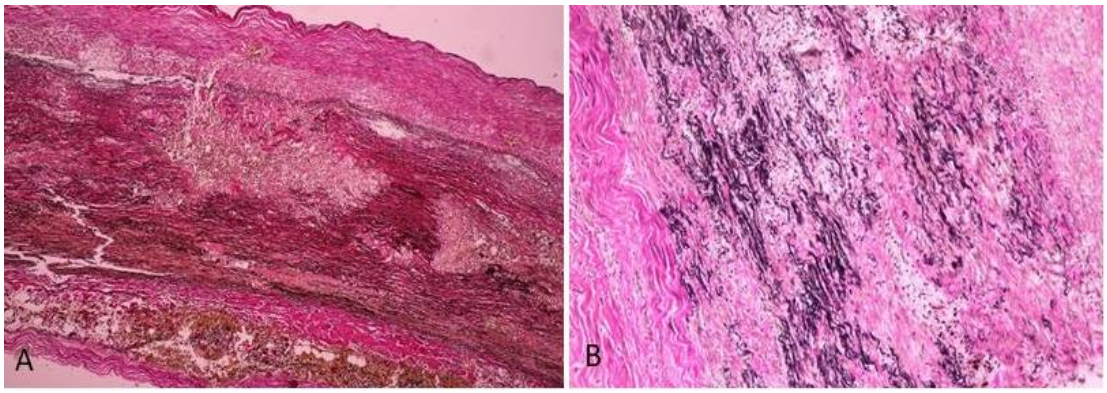

Figure I.2.3. (A) GCA aortitis – micronodular granulomas on the inner half of the media and reduced adventitial fibrosis (EVG, x100); (B) Takayasu aortitis – medial GC granuloma and increased adventitial fibrosis (EVG, x200).

The common histological features found in granulomatous aortitis differed according to their etiology. (a) In GCA were identified: diffuse intimal hyperplasia; granulomatous

inflammation of the media that comprised epithelioid macrophages and occasional giant cells directly related to “laminar medial necrosis”; areas of smooth muscle cell loss with collapsed elastic fibers; fibrotic scars and associated adventitial lymphoplasmacytic infiltrate (fig. I.2.3.A). (b) In TA, histological examination indicated: diffuse intimal hyperplasia, compact granulomas and necrosis in the tunica media, together with medial scarring, disruption and disorganization of the remaining elastic fibers, and dense adventitial fibrosis (fig. I.2.3.B).

Among these five aortitis case reports, one was diagnosed as syphilitic aortitis on the basis of the pathological features and patient's history. The disease was identified in a male in his fifth decade, who presented valvular regurgitation, aneurysmal disease, and myocardial ischemia.Therefore he needed CABG surgery. All these lesions were cardiovascular complications of syphilis, involving both the aorta (leading to the formation of aneurysms and aortic-valve incompetence) and the coronary arteries, and causing coronary ostial stenosis (angina pectoris complaints). Histopathological examination revealed a chronic mesoaortitis, characterized by the media replacement with fibrous scars and adventitial endarteritis of the vasa vasorum (fig. I.2.4).

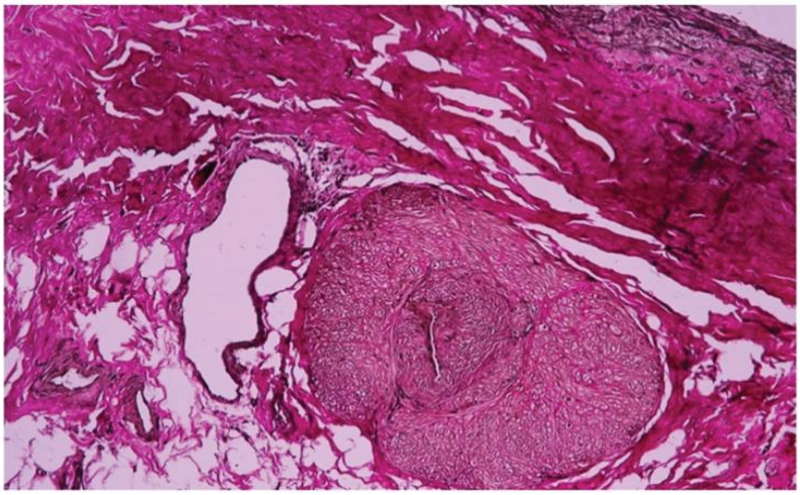

Figure I.2.4. Endarteritis obliterans of the vasa vasorum and medial aortic scar. Elastic Van Gieson's staining, x 400.

I.2.4. Discussion

An aortic aneurysm is defined as a dilation of an aortic segment with a diameter of at least one and one-half times the normal diameter (Elefteriades et al, 2002), involving the ascending aorta or descending aorta. In our study, the aortic aneurysms included 39 (75%) thoracic and 13 (25%) abdominal cases.

Other epidemiology studies reported that AAA involves mainly older patients while TAA involves mainly two populations: one of old age with no special history (Fritze et al., 2011) and other one of young age suffering from Connective Tissue Disease (CTD), including Marfan's syndrome (MS)(Iams et al, 2010). We did not find any MS or CTDs in our young patients with TAA. In this respect, we would like to point out the importance of a good family history, histopathological examination and genetic screening.

The most common risk factors for the aortic aneurysm development are: advanced age (over 65 years old), male gender, smoking, high blood pressure and family history (Hiratzka et al, 2010). Our results confirmed these statements. The potential risk factors identified by us to be involved in AA development were advanced age, hypertension and bicuspid aortic valve disease in TAA and advanced age, male gender, smoking and ATS in AAA.

Previous studies reported that TAAs are most often associated with medial degeneration (MD), which can be regarded as a structural alteration responsible for the development of aortic aneurysm in relation to other risk factors (Leone et al, 2012).

In our study, the histopathological profile showed: (a) medial degeneration was the most common histopathological substrate in TAA (64.1%); (b) granulomatous aortitis (10.25%) was a rare cause of TAA, and syphilitic aortitis (2.5%) was a very rarely encountered case; (c) ATS was the common histopathological substrate in AAA (100%).

In patients with AAs, histological analysis of the aortic resection specimens provides important diagnostic information on the MD lesions, describing a definite pattern of distribution of MD severity within the aortic wall. Moreover, aortitis was an unexpected diagnosis through histological examination of surgically resected aortic thoracic aneurysms in persons without clinical signs or symptoms of systemic vasculitis. Aortic thoracic aneurysm is in most cases a fatal condition, especially in association with more severe degenerative changes in the aortic media.

Giant cell arteritis (GCA) and Takayasu's arteritis, diagnosed in our cases, are considered the most common causes of noninfectious aortitis. At least 20% of patients with GCA and 50% of patients with Takayasu's arteritis would develop changes consistent with aneurysmal aortitis, while we found that only 7.6% of patients with GCA had TAA and 2.5% patients with Takayasu's arteritis had TAA, the remaining one TAA case having syphilitic aortitis (Garcia-Martinez et al, 2008).

Histopathological examination is not sufficient to distinguish between the two conditions, and, together with clinical data, it must be integrated within the diagnosis algorithm. In our final diagnosis we applied American College of Rheumatology (ACR) criteria and we identified at least three ACR criteria, including clinical, imagistic and histological data, which were enough to confirm the two granulomatous aortitis (Park et al, 2005; Ness et al, 2013).

We found syphilitic aortitis to be a rare cause of AA from all TAA cases. The complications in the form of aortic aneurysm and insufficiency in association with coronary ostial stenosis frequently coexist in the tertiary syphilis (Vaideeswar et al, 2013). For diagnosis, we have to take in consideration not only the pathological findings but also clinical features and serology.

Our study also included 20 cases of atherosclerotic lesions. Clinical analysis of the 20 AAs revealed two types of aneurysmal diseases, 7 (35%) mixed thoracic aortic aneurysms and 13 (65%) ATS abdominal aortic aneurysms. We also noted that aortic atherosclerosis accounted for most abdominal aortic aneurysms (Singh et al, 2001). In our cases, the histopathological examination revealed intimal atheroma and parietal thrombosis in both AAA and aortic atherosclerosis, similarly with CVD consensus group observations (Stone et al, 2011). The presence of ATS lesions in both groups may suggest the need for ATS medical therapy, in all TAA and AAA patients. Due to the highest degree of severity of the ATS AAA lesions, we consider that atherosclerosis plays an important role in AAA development.

Final remarks

Our study provides evidence of inclusion of histopathological examination in final diagnosis, in consensus with the late knowledge and classification of aortic surgical pathology for diagnosis optimization.

Prospective studies including a large study group will be necessary to evaluate histologically the extent and severity of disease and disease progression.

I.3. Immunohistochemical interplay in giant cell aortitis in patients with consequent ascending aortic aneurysm

I.3.1. Introduction

Giant cell arteritis is characterized by immune-mediated injury of predominantly large vessels, including aorta. Histopathologic lesions contain mononuclear cell infiltration of the vessel wall that often includes granuloma formation. Within this group of diseases, Takayasu's arteritis (TA) affects younger individuals (under 50 years), whereas giant cell arteritis (GCA) is common in patients over 50 years. Both giant cell arteritis predominantly affect women (Jennette et al, 2013). It has been suggested that GCA and TA may be the same disease with the same etiology, but with phenotypic variations due to immune and aortic aging substrate (Maksimowicz-McKinnon et al, 2009; Grayson et al, 2012). The diagnosis is difficult because aortitis rarely has specific symptoms and diagnosis is made often by histopathology. The occurrence of concomitant aortitis and AAA are common forms of presentations in both diseases (Maffei et al, 2006; Chacko et al, 2015). Giant cell arteritis and TA are two granulomatous vasculitis which develop on a specific genetic background and share some similarities regarding the immunological pathways involved in their pathogenesis (Samson et al, 2016). Generally, it is supposed that aortitis is the result of stimulation from an antigen of unknown nature, possibly infectious type. It seems that GCA could be initiated by exogenous antigens like viruses and bacteria, including parainfluenza virus, varicella zoster virus, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* (Gilden et al, 2015). Similarly, TA patients had an increased immune response to *Mycobacterium tuberculosis* (Moraes et al, 1999). In addition, it might be initiated in the adventitia, with inflammatory cells entering through the vasa vasorum and subsequently infiltrating into all layers of the aortic wall (Samson et al, 2016).

Objective

This study focused on the defining characteristics of the two inflammatory aortopathies, specifically those affecting the ascending aorta, and discusses the important clinical and pathological distinction between them. In our analysis we compared IHC results of the two cases of giant cell aortitis, in order to a better understanding of these pathologies.

I.3.2. Material and methods

The two cases of TA and GCA patients were one female under 50 years old and one male over 50 years old, respectively. The study material, the residual histological blocks, was used for histological and IHC comparative examination. These two cases were selected from 10 cases of ascending TAA associated with aortitis, both having at least three positive criteria of diagnosis according to ACR aortitis classification.

We analyzed the aortic morphology on hematoxylin-eosin (HE) and elastic tissue fibers on Verhoeff's Van Gieson (EVG) sections by using an Olympus microscope (model CX41; Olympus, Tokyo, Japan). Morphometric analysis of macrophages (CD68), T-lymphocytes (CD3) and B-lymphocytes (CD20) was accomplished with imaging analysis software (Quick Photo Micro 3.0).

Histologically we analyzed 10 histological fields at a magnification of x200, for each case. We measured the aortic wall thickness. The measurements were expressed as mean values.

We analyzed immunohistochemically 10 histological fields at a magnification of x200 for each case, too. The degree of total wall inflammation was evaluated by relating the average inflammatory cell number to the entire histological section area studied. Data were expressed as mean values.

For the evaluation of each inflammatory cell component of the inflammatory infiltrate we calculated the proportion of each cell number related to the total inflammatory cell number in the same histological section area. The results were expressed as percentages.

The inflammatory degree of the macrophages, B and T cells was morphologically classified by inflammatory cell distribution in the aortic tissue, as follows: grade 0 (<10%), grade 1 (between 10-50%), grade 2 (between 50-75%), and grade 3 (> 75%). The end inflammatory score was the sum of the score for each type of inflammatory cell. Finally, we compared the score results.

IHC exam enabled the comparison between the composition and distribution of the inflammatory infiltrate of the ascending TAA wall, in GCA and TA cases, respectively. Results were statistically processed. Data were expressed as mean values and percentages, calculated using Excel software (Microsoft Corporation, Redmond, WA, USA).

I.3.3. Results

The average values of aortic thickness (intima, media and adventitia) in the GCA and TA are presented in table I.3.1. The aortic wall thickness almost doubled in TA (4034 μm) in comparison with GCA (2122 μm) values.

Histologically, in GCA we found an intimal fibrous extracellular matrix containing foci of mononuclear inflammatory cells mainly located at I-M junction, medial fibrous areas with nodular and band-like inflammatory foci in association with neovascularization, and adventitial nodular and band-like inflammatory foci and intense neovascularization (fig. I.3.1.a).

In TA, we noted a thickened intima by collagenous bands disposed parallel to the endothelium, medial areas of elastic lamina destruction and nodular and band-like inflammatory foci along with neovascularization, and thickened adventitia due to connective extracellular matrix and neovascularization (fig. I.3.1.b).

Distribution of inflammatory cells within aortic layers, in giant cell arteritis (GCA) and Takayasu’s arteritis (TA) are showed in table I.3.1.

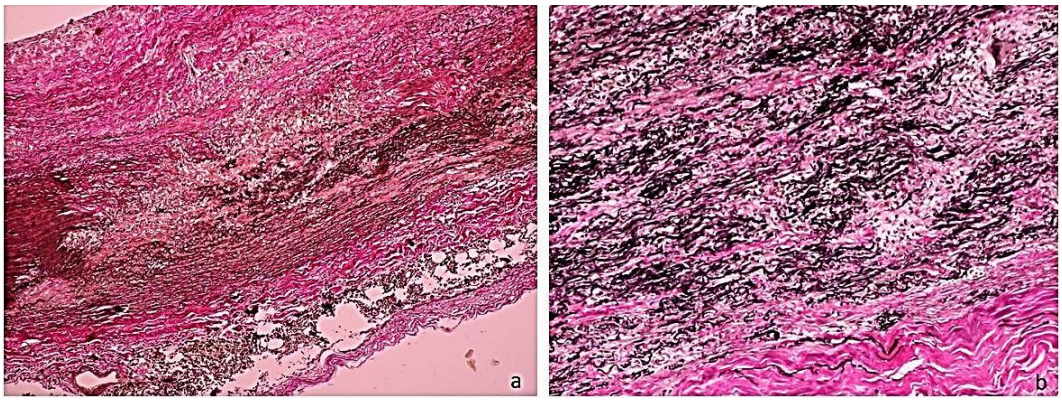

Figure I.3.1 a. Giant cell arteritis aortic wall x100 EVG (elastic tissue fibers - Verhoeff's Van Gieson); b. Takayasu's arteritis aortic wall x200 EVG.

In the aortic wall, inflammatory cells are 1.16 times more numerous in GCA (1117) than in TA (958), and have different values in the different layers of the aortic wall. Inflammatory infiltrate is 11.6 higher in the intima of the aorta in GCA (221) than in TA (19) and only 1.79 times higher in aortic adventitia in GCA (620), than TA (345).

Inflammatory cell ratio was approximately double (2.15) in the media of the aortic wall in TA (594) when compared to GCA (276) or, in other words, inflammatory cell ratio in GCA was less than half their TA value.

TABLE I.3.1. Assessement of the aortic wall thickness and inflammatory cells in giant cell arteritis (GCA) and Takayasu's arteritis (TA)

	GCA			TA			IR
	Thickness µm		N.I.C.	Thickness		N.I.C.	GCA/TA
	No	%		No	%		
Intima	546	0.26	221	662	0.17	19	11.63
Media	1045.5	0.49	276	1405	0.35	594	0.46
Adventitia	530.5	0.24	620	1967	0.48	345	1.79
Total	2122	100%	1117	4034	100%	958	1.16

NIC: number of inflammatory cells; IR: inflammation ratio=NIC GCA/NIC TA

Consequently, our results showed that both aortitis are panarteritis, with similar histology and different distribution and degrees of inflammation within the aortic wall. In our opinion, inflammation is a chronic phase process in both aortitis, yet with persistent manifest inflammation in TA. The proportion of inflammatory cells within aortic wall layers is showed in table I.3.2.

In GCA, the inflammatory infiltrate (1117 cells) contained 39.3% T - lymphocytes (CD3), 22.4% B - lymphocytes (CD20) and 38.3% macrophages (CD68), while in TA, the inflammatory infiltrate (958 cells) contained 61.5% T - lymphocytes (CD3), 22.8% B - lymphocytes (CD20) and 15.7% macrophages (CD68).

TABLE I.3.2. Proportion of inflammatory cells within aortic wall layers in giant cell arteritis (GCA) and Takayasu's arteritis (TA)

GCA & TA-IMH (number, %)	CD3	CD20	CD68	Total N.I.C.
GCA				
Intima cells (%)	21	8.5	70.5	221
Media cells (%)	39.2	8.6	52.2	276
Adv. cells (%)	45.7	33.5	20.8	620
Total aortic wall cell (%)	39.3	22.4	38.3	1117
TA				
Intima cells (%)	5.26	-	-	19
Media cells (%)	60.2	28.2	11.6	594
Adv. cells (%)	61.1	14.9	24	345
Total aortic wall cells (%)	61.5	22.8	15.7	958

GCA: giant cell arteritis; IMH: immunohistochemical; TA: Takayasu's arteritis.

Comparative inflammatory cell layer distribution in GCA vs TA was the following:

• In GCA, intima contains 221 mononuclear inflammatory cells, of which 21% are T - lymphocytes, 8.5% are B - lymphocytes and 70.5% are macrophages while in TA, all 19 intimal inflammatory cells were T - lymphocytes only.

• The medial layer contains in GCA 276 mononuclear inflammatory cells, of which 39.2% are T - lymphocytes, 8.6% are B - lymphocytes and 52.2% are macrophages, while in TA of 594 mononuclear inflammatory cells, 60.2% are T - lymphocytes, 28.2% are B - lymphocytes and 11.6% are macrophages.

• In GCA, adventitia contains 620 mononuclear inflammatory cells, of which 45.7% are T - lymphocytes, 33.5% are B - lymphocytes and 20.8% are macrophages, while in TA of 345 mononuclear inflammatory cells, 61.1% are T - lymphocytes, 14.9% are B - lymphocytes and 24% are macrophages.

Entire aortic wall inflammatory score in GCA and TA is showed in table I.3.3. Total wall inflammatory infiltrate score was bigger in TA (4) than in GCA (3).

Individual inflammatory cell score in GCA and TA is showed in table I.3.4. The inflammatory infiltrate had a similar distribution in the aortic all, with different proportion of inflammatory cells (fig. I.3.2).

TABLE I.3.3. Total wall inflammatory score in GCA and TA

NIC	T - Lys (%)	Grade	B- Lys (%)	Grade	Macrophages (%)	Grade	Sum Score
GCA (N=1117)	39.3	1	22.4	1	38.3	1	3
TA (N=958)	61.5	2	22.8	1	15.7	1	4

GCA: giant cell arteritis; Lys: lymphocytes; TA: Takayasu's arteritis.
NIC: number of inflammatory cells; score = grade sum.

TABLE I.3.4. Inflammatory cell score in giant cell arteritis (GCA) and Takayasu's arteritis (TA) within aortic wall layers

GCA and TA inflammatory cell % & score	CD3 %-score		CD20 %-score		CD68 %-score		Total score
GCA							
Intima	21	1	8.5	0	70.5	2	3
Media	39.2	1	8.6	0	52.2	2	3
Adventitia	45.7	1	33.5	1	20.8	1	3
Total GCA IWS		3		1		5	9
TA							
Intima	5.26	0	-	-	-	-	0
Media	60.2	2	28.2	1	11.6	1	4
Adventitia	61.1	2	14.9	1	24	1	4
Total TA IWS		4		2		2	8

Inflammatory wall score: IWS

Comparative inflammatory score in GCA versus TA was assessed in accordance with two criteria. Depending on the aortic wall layers involved, the total inflammatory score in GCA was 9, the same in intima (3), media (3) and adventitia (3), while the total inflammatory score in TA was 8, having 0 value in intima and 4, in both media and adventitia. Depending on the type of inflammatory cells in the entire wall thickness, the inflammatory score in GCA was 3 for T – lymphocytes (CD3), 1 for B - lymphocytes (CD20) and 5 for macrophages (CD68), while the inflammatory score in TA was 4 for T – lymphocytes, 2 for B - lymphocytes and 2 for macrophages.

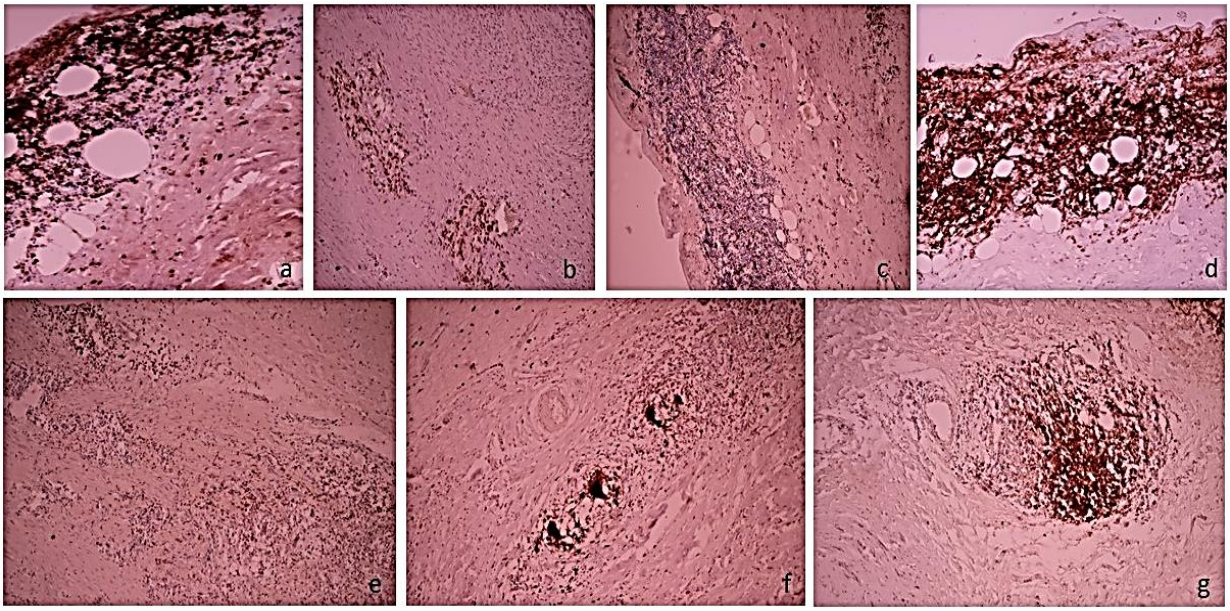

Figure I.3.2. ***Giant cell arteritis****: a-adventitial CD3; b-medial CD3; c-medio-adventitial CD68; d-adventitial CD20.* ***Takayasu's arteritis****: e-intimo-medial CD3; f-medio-adventitial CD68; g-adventitial CD20.*

In GCA, the proportion of the inflammatory cells of 3:1:5 probably denotes a chronic healing process with a recent mild activation by an exogenous agent, may be a viral one.

In TA, the proportion of the inflammatory cells of 4:2:2 probably denotes a persistent chronic inflammation which is still active (due to persistence of antigen stimulation correlated with tuberculosis history), demonstrated by extensive destructive areas.

Both aortitis have approximately the same inflammatory score, but they are in various evolutive stages related to the different intensity of inflammatory activity.

I.3.4. Discussion

Takayasu arteritis and GCA are the two main variants of large vessel vasculitis (LVV). Takayasu's arteritis is a LVV affecting elastic arteries such as the aorta and its branches, while GCA refers to involvement of the aorta and its proximal or extracranial aortic branches. In a patient with GCA, the aortic involvement is revealed by using computed tomography angiography (angio-CT) and aortic biopsy (Prieto-Gonzalez et al, 2012).

These two conditions have been considered separate entities because of the differences observed in the age at onset, their clinical features, geographic distribution and location of arterial involvement (Maksimowicz-McKinnon et al, 2009). Authors noted that TA and GCA may exist within the clinical spectrum of a single disease (Polachek et al, 2015).

Both our cases had ascending TAA and aortic insufficiency at hospital presentation. Both LVV cases had positive inflammatory markers and angiographic signs of ascending aortic involvement, completed with specific signs of aortic branch stenosis in TA case. The artery biopsy was the gold standard for diagnosis of these aortitis.

As other researchers (Eberhardt et al, 2007; Burke et al, 2007), we found at GCA histology exam a chronic evolutive stage of the GCA characterized by moderate intimal thickening due to fibromyxoid changes, medial granulomas with rare multinucleated giant cells and medial and adventitial fibrosis. Inflammation showed to be intense in the adventitia, too.

We also reported, as other scientists did (Vaideeswar et al, 2013), a chronic evolutive stage at the histological TA exam, consisting in thicker, collagenous intima, medial focal granulomas related to elastic fibers fragmentations and medial fibrosis, and focal adventiceal perivasa vasorum inflammatory infiltrate. In addition, we also noted neovascularization within the entire wall.

Due to similar GCA and TA histopathological findings, only the biopsy results may not differentiate between the two vasculitides. Hence, in discriminating between the two aortitis, the age in both and CT angiography in TA are essential tools.

Similarly to other results (Chakravarti et al, 2015), we consider that more severe medial inflammation leads to loss of smooth muscle cells and elastic fibers, medial weakening and aneurysm formation.

In both TA and GCA, there are parietal inflammatory lesions produced in the adventitia through the vasa vasorum, allowing the lymphocytes to gain access to the arterial wall (Noris et al, 2001). Indeed, we also observed mononuclear inflammatory cells forming a vasa vasorum ring and spilling within the aortic wall.

Cell-mediated autoimmunity has been clearly involved in the pathophysiology of vascular cell injury in TA and GCA, due to the highest values of T - lymphocytes and macrophages in these two diseases. We did not find GCA associated infections (virus infection cannot be excluded) while we noted a history of tuberculosis infection in the TA patient. Furthermore, an increased immune response to *Mycobacterium tuberculosis* has been reported

in TA cases (Moraes et al, 1999). We consider, that using lymphocytic and histiocytic markers in suspected GCA and TA we can detect residual arteritis in patients with resolving disease.

If humoral immunity takes part in the pathophysiology of GCA and TA is not clear. We found more B Lys in GCA than TA, and we consider that both aortitis have a persistent active inflammation of different degrees.

LVV adventitial analysis showed that inflammation was most prominent in GCA, with infiltration of B and T cells. In TA, we found adventitial inflammatory foci, with B and T cells surrounding vasa vasorum. The distribution of immunostainings seen at the outer media border indicates that the majority of the inflammatory cells enters the arterial wall from adventitial microvessels, migrate through the media and the inflammatory activity focuses finally on the media and intima.

Final remarks

This study showed that the initial site of inflammation seems to be around the adventitial vasa vasora which is an early stage in the development of classic transmural inflammatory infiltration. The medial inflammatory areas related to prominent elastic fibers fragmentation was the cause of medial weakening and aneurysm formation.

CHAPTER II. POSTOPERATORY ATRIAL FIBRILLATION: FROM ETIOLOGY TO DIAGNOSIS

II.1. State of the art

Atrial fibrillation (AF) is the most common arrhythmia. AF occurs and maintains itself in the context of a morphologically and functionally altered atrial substrate that can be induced by stressors such as underlying diseases (cardiac or noncardiac) or aging. Potential risk factors, such as coronary artery disease, kidney disease, systemic inflammation, pericardial fat, hypertension, valve disease, heart failure, myocardial infarction, obesity, diabetes mellitus, thyroid dysfunction, alcohol consumption and tobacco use, all cause structural remodeling (Almassi et al, 1997; Hogue et al, 2000). The resultant structural remodeling is a slow process that progressively affects myocytes and the myocardial interstitium. The mechanisms that underlie the remodeling process in atrial fibrillation have not yet been completely elucidated, although experimental and clinical investigations have indicated a number of signaling systems, inflammation, oxidative stress, atrial stretching and ischemia as factors involved in the cascade of events that leads to atrial fibrillation (Corrado et al, 2008). Better recognition of the clinical epidemiology of AF, as well as an improved appreciation of the underlying structural changes, is needed to develop improved methods for AF diagnosis and management (Zaman et al, 2000).

There are limited treatment strategies for prevention of disease onset and progression. Development of novel therapies for primary and secondary prevention of AF is critical and requires improved understanding of the cellular and molecular mechanisms underlying the AF disease process. Translational and clinical studies conducted over the past twenty years have revealed that atrial remodeling in AF shares several important pathophysiologic traits with the remodeling processes exhibited by hibernating myocardium that develop in response to chronic ischemia. These shared features, which include an array of structural, metabolic, and electrophysiologic changes, appear to represent a conserved adaptive myocyte response to chronic stress that involves dedifferentiation towards a fetal phenotype to promote survival. Ultimately, better understanding of the molecular mechanisms of atrial myocyte remodeling during the onset of AF and the transition from paroxysmal to persistent stages of the disease may facilitate discovery of new therapeutic targets.

The pathophysiology of AF is complex and often multifactorial, generally involving an electroanatomical substrate, abnormal impulse formation and/or propagation, focal and dynamic triggers, reentry, and fibrosis in atrial myocardium. While recent studies have identified a familial form of AF (Lubitz, 2010), the majority of patients with AF have the common form with no defined genetic susceptibility. Nowadays, the pathophysiology of AF is centered on structural remodeling, that occurs throughout the disease process.

Atrial morphological changes form a structural substrate for AF. Several characteristic atrial architectural changes are typically observed in preclinical models of AF and in patients suffering of disease. These include inflammation, cell hypertrophy, atrial dilation, and fibrosis, which cumulatively contribute to abnormal electrical signal formation and conduction as an arrhythmogenic substrate (Allessie et al, 2001). These changes are commonly the result of other underlying heart diseases, such as coronary artery disease,

hypertension, valvular disease, and cardiomyopathies, which exert adverse effects on myocyte structure and/or function, predominately via elevations in atrial pressure and wall stress (January et al, 2014). The elevated hemodynamic load on the atria promotes cellular hypertrophy, cardiomyocyte dysfunction, and disorganization of gap junctions. This process is associated with myocyte death through apoptosis and necrosis. Myocyte loss, together with neurohumoral signaling activated by atrial stretch, prompts extensive replacement fibrosis in large part because of the greater number of fibroblasts present in the atria and its subsequent propensity for fibrotic tissue deposition (January et al, 2014).

Collectively, the aforementioned morphological changes contribute to the formation of a structural substrate that promotes the onset and progression of AF. For instance, loss of atrial cardiomyocyte mass with apoptosis or necrosis causes accumulation of fibrotic tissue, disruption of cell-to-cell communication, diminished conduction velocity, and heterogeneous conduction patterns (Allessie et al, 2001; Nattel et al, 2002; Wakili et al, 2011). This replacement fibrosis, along with amyloidosis, inflammation, and remodeling of the extracellular matrix, disrupts gap junctions and impairs cell coupling (Aviles et al, 2013; Burstein and Nattel, 2008; Nishida et al, 2010). Moreover, the concomitant presence of obesity and metabolic risk factors may exacerbate this process via the release of proinflammatory mediators from epicardial fat (Batal, 2010; Chilukoti, 2015).

Regardless of the initiating stimulus, paroxysmal AF promotes further AF and stretch, leading to more extensive fibrosis and increased extracellular matrix deposition that ultimately slows or blocks intra-atrial conduction as a part of the adaptation to high atrial rates. This high and variable rate of atrial contraction may cause significant metabolic and oxidative stress as seen in tachyarrhythmias, and microcirculatory flow abnormalities in the left ventricle (Bukowska, 2012). Ultimately, these flow abnormalities and structural changes that initiate AF are worsened by AF itself (i.e., "AF begets AF"), forming a vicious cycle that heralds progression of disease severity (Wijffels et al, 1995).

Cardiomyocyte cellular and molecular remodeling in atrial fibrillation. Initial insight into the cellular structural remodeling associated with chronic AF was noted by researchers in "The American Journal of Pathology" (Thiedemann and Ferrans, 1997). They used light and electronic microscopy to demonstrate fibrosis, myocyte hypertrophy, and myolysis in atrial tissue samples from patients with AF secondary to mitral valve disease. Several years later, these findings were supported in a canine model of mitral valve fibrosis characterized by left atrial enlargement and the common occurrence of atrial arrhythmias (Boyden, 1982). These animals exhibited reduced atrial wall thickness and substantial connective tissue between notably hypertrophied myocytes organized into disarranged cell bundles. More recent studies, in patients with AF (Aimé-Sempé et al, 1999; Ausma, 2001), extended these observations and shown sarcomere depletion and glycogen accumulation in remodeled atrial myocytes, although the concomitant presence of valve disease in the majority of subjects may have contributed to these results.

To address this limitation, experimental animal models of lone AF have proven useful to delineate patterns of structural remodeling in myocytes after prolonged periods of AF. For example, rapid atrial pacing-induced AF in dogs elicited characteristic changes including structural alterations consisting of atrial chamber dilation and myocyte hypertrophy (Morillo et al, 1995). This approach was translated to a goat model (Wijffels et al, 1995), in which

animals exhibited enlarged atrial myocytes and glycogen accumulation that progressively worsened with increasing disease severity (Ausma and Borgers, 2002). In addition, disorganization of sarcoplasmic reticulum, appearance of mini-mitochondria, reductions in T-tubular sarcolemmal invaginations, and dispersion of nuclear chromatin were observed (Ausma et al, 1997). Interestingly, many of the atrial cardiomyocytes reacquired characteristics of fetal cardiomyocytes, including expression of α-smooth muscle actin, loss of cardiotin, and a punctuated titin staining pattern (Ausma, 1997). It was concluded that AF was associated with myocyte dedifferentiation in the absence of degeneration, perhaps representing a conserved cellular response to stress. A more recent study (Barth et al, 2005) provided additional insight into this remodeling process in AF patients. Using a genome-wide approach to compare atrial mRNA expression in AF patients versus patients with sinus rhythm, the authors identified over 1400 genes that were deregulated in chronic AF. Functional classification analysis revealed a pattern of remodeling consistent with prominent fibrosis and metabolic adaptation to long-term metabolic stress, including upregulation of genes related to extracellular matrix composition, downregulation of contractile proteins, and coordinated transcriptional changes in metabolic enzymes favoring a shift from fatty acid oxidation to glucose utilization (Barth et al, 2005). Additionally, atrial tissue from AF patients exhibited a general "ventricularization" characterized by substantial upregulation of ventricle-predominant genes and downregulation of atrial-specific genes, consistent with myocyte dedifferentiation and adoption of a fetal phenotype aiming at improving cell survival during extended periods of stress (Tomaselli et al, 2005).

Collectively, these results show that, aside from the atrial structural and electrical remodeling that occurs in AF, cardiomyocytes that do not succumb to apoptosis or necrosis undergo several characteristic cellular and molecular phenotypic changes. Interestingly, these alterations are reminiscent of those observed during cell dedifferentiation, including an increase in myocyte volume, myolysis, glycogen accumulation, mitochondrial changes, and chromatin redistribution (Thijssen et al, 2001). Because these changes commonly occur in the absence of clear signs of degeneration, it has been suggested that atrial myocyte dedifferentiation in AF represents an adaptive, programmed cell survival response. While this idea is supported by findings in several experimental models of AF, clinical studies of AF patients that exhibit cardiomyocyte degeneration argue against this notion. These divergent observations may implicate comorbidities and/or the longer duration of disease in patients as primary factors determining the adaptive versus degenerative nature of myocyte remodeling in AF (Thijssen et al, 2001). Nevertheless, our current knowledge in this area supports the notion that myocyte remodeling in AF (particularly in patients with lone AF) represents an adaptive response to stress that aims to conserve energy and promote survival, similar to what has been observed in other conditions of myocardial stress, such as the development of hibernating myocardium (HM) in response to chronic ischemia. Therefore, these disease states may facilitate the development of novel therapeutic approaches by encouraging a shift in our perspective to a position that recognizes the adaptive, and potentially reversible, nature of myocyte remodeling during chronic stress.

Therefore, our studies were focused on clinical, etiological and histopathological features of postoperative atrial fibrillation (POAF) in comparison with those encountered in patients remaining in sinus rhythm (SR).

The interest in this field lead to results published in 6 scientific papers (1 in journals indexed in international databases, 5 in journals indexed in Clarivate Analytics). The papers published in journals with impact factor (Clarivate Analytics) are presented below:

1. **Butcovan D**, Lupusoru CE, Baran D, Cimpeanu C, Jelihovschi I, Ursu RG, Haliga RE, Lupusoru RV. The Interplay between Cardiomyocytes and Non-cardiomyocytes in Postoperative Atrial Fibrillation. *Rev Chim* 2016; 67(10): 2012-2014. **IF=1.232**
2. **Butcovan D**, Jelihovschi I, Baran D, Ivan L, Cimpeanu C, Lupusoru CE, Haliga RE, Lupusoru RV. Atrial Structural Remodeling in Coronary Patients with and without Postoperative Atrial Fibrillation. *Rev Chim* 2016; 67(9): 1797-1799. **IF=1.232**
3. **Butcovan D**, Oboroceanu T, Cimpeanu C, Mironescu A, Haliga RE, Pinzariu AC, Lupusoru RV, Popescu E, Mocanu V. The Involvement of Epicardial Adiposity and Inflammation in Postoperatory Atrial Fibrilation - Immunohistochemical Qualitative and Quantitative Assessment. *Rev Chim* 2017; 68(4): 886-889. **IF=1.412**
4. Tinica G, Mocanu V, Zugun-Eloae F, **Butcovan D**. Clinical and histological predictive risk factors of atrial fibrillation in patients undergoing open-heart surgery. *Exp Ther Med* 2015; 10(6): 2299-2304. **IF=1.280**
5. Timofte D, Mocanu V, Zugun-Eloae F, Hristov I, Cretu IS, Aursulesei V, Balan GG, Ciuntu BM, Oboroceanu T, Tiron A, Costan VV, **Butcovan D**. Immunohistochemical Expression of Growth Hormone Secretagogue Receptor (GSH-R) of Adipose Tissue Macrophagesin Obese Bariatric Patients. *Rev Chim* 2019; 70(9): 3428-3430. **IF=1.605**

II.2. Etiopathological profile of the fibrillating heart

II.2.1. Introduction

Atrial fibrillation (AF) is a common complication after cardiac operations, occurring within the first week postoperatively in 20% to 40% of the patients undergoing coronary artery bypass grafting (CABG) and valvular surgery (Hogue et al, 2000). Although POAF is often regarded as a temporary problem, this complication has significant adverse effects: it increases the risk of cerebrovascular accident and extends intensive care unit and hospital stay (Almassi et al, 1997).

Recognizing patients at high risk for developing postoperative atrial fibrillation (POAF) may help identify those who could benefit from strategies to prevent POAF.

Advanced age is the most consistently reported and widely accepted risk factor for POAF. The aging process leads to a loss of myocardial fibers, increased fibrosis and collagen deposition in the atria, particularly near the sinoatrial node, which alters atrial electrical properties (Mitchell, 2011).

Cardiovascular risk factors for POAF include prior history of AF or other arrhythmias, non-coronary vascular disease, congestive heart failure, coronary artery disease, hypertension, left atrial enlargement and left ventricular dysfunction (Kramer et al, 2015).

Non-cardiovascular risk factors for POAF include: male gender, chronic obstructive pulmonary disease, high cholesterol, hyperthyroidism, chronic kidney disease, diabetes, obesity and greater body surface area. Individuals with a high body surface area (around 2.0 m^2) often have larger atria and abnormal intrathoracic pressure, which may alter atrial electrophysiological properties and increase susceptibility to POAF (Svagzdiene & Sirvinskas, 2006).

According with many researches, the most common risk factors associated with development of POAF include advanced age, prolonged preoperative atrial conduction duration, atrial myocardial ischemia, prolonged aortic cross-clamping time, and right atrial manipulation (Zaman et al, 2000).

Taken together, these factors indicate that preoperative status of the patient and the atria are major determinants of the risk to develop POAF, in addition to the stress and ischemia induced by the surgical procedure per se.

The pathogenesis of POAF is a multifactorial process, which involves patient-related factors, cardiac status, inflammation, electrolyte status, and operation method. Currently, a risk profile obtained from cardiac subjects has been employed as a reference to predict and assess the risk of POAF in cardiac patients with CABG.

So, of great interest is to establish the risk profile of adult patients undergoing isolated CABG or valve diseases in Iasi County, taking in consideration multiple variables as potential risk factors.

Objective

The present study was designed to correlate clinical features and any atrial lesions with the occurrence of POAF, and thus to identify markers for an increased vulnerability in developing atrial fibrillation after CABG or valve surgery.

II.2.2. Material and Methods

Samples of right atrial appendage (RAA) tissue were taken from 103 patients in normal sinus rhythm undergoing CABG (82) and valve surgery (21), before cardiopulmonary bypass (CBP). The age of the patients ranged from 42 to 77 years (mean, 61.7+14 years). 76 of the patients were women (73.78%).

Atrial specimens were fixed in 10% buffered formalin for 12 hours at room temperature and then were routinely dehydrated, embedded in paraffin, and cut into 4-6 mm serial sections. Sections were stained with hematoxylin-eosin and Masson trichrome.

The specimens were examined histologically by using a previous developed standard protocol for the examination of the RAA (tab. II.2.1). We modified the standard protocol by adding the endocardial analysis, as well.

An Olympus CX41 light microscope (Olympus, Tokyo, Japan) was used for morphological evaluation. Pathology referring to atrial myocytes and connective tissue components were evaluated by using semiquantitative scales similar to that previously described by Ad and colleagues (tab. II.2.1).

Fibrosis was evaluated at the level of the endocardium. In cardiomyocytes (CMs), examination included the assessment of the degree of myocytolysis (loss of muscular striations and displacement of cell content by cytoplasmic vacuoles), the existence of atrophy and muscle disarray, and the existence of apoptotic figures or other degenerative cell lesions. In atrial interstitium, we assessed the degree of fibrosis and arteriolar hypertrophy. We didn't find any interstitial inflammatory lesions including interstitial edema and mononuclear exudates. In the pericardium, we looked after the existence of mononuclear cells or fibrinous exudates, adiposity and fibrosis.

All patients were monitored daily until discharge with continuous electrocardiographic recording with standard 12-lead electrocardiography. Additional recordings were collected at clinical suspicion of AF. Only AF episodes lasting longer than 15 minutes were considered. Patients were considered to have POAF if interventional therapy (drugs or electrical cardioversion) was required to restore sinus rhythm.

TABLE II.2.1. Modified Protocol for Routine Histopathological Examination of the RAA specimens (after Ad N et al, 2001)

Endocardium
- Endocardial fibrosis: Absent / Present
- Mononuclear exudates: Absent / Present

Myocardium
- Myocytes
  - Myolytic vacuolation: Absent / Present
    If present:
    a. Size of vacuoles: Mild / Severe
    b. Frequency: 25%; > 25%
  - Hypertrophy: Absent / Present
  - Atrophy: Absent / Present
  - Lipofuscin: Absent / Present
  - Abnormal nuclei: Apoptotic figures
  - Contraction band necrosis: Absent / Present
- Interstitial myocardium
  - Interstitial fibrosis
    - Amount: Mild / Severe
    - Frequency: 25%; > 25%
  - Perivascular fibrosis: Absent / Present
  - Arteriolar medial hypertrophy: Absent / Present

Pericardium
- Mononuclear exudates: Absent / Present
- Pericardial adiposity: Absent / Present
- Pericardial fibrosis: Absent / Present

Statistical analysis was assessed by correlating the histological variables from patients with postoperatory sinus rhythm (POSR) and POAF using chi test. A *p* value less than 0.05 was considered significant.

II.2.3. Results

POAF was seen in 37 of 103 patients (35.92%), which occurred at 12 to 144 hours after surgery. The average age of the patients with POAF was higher (61.7+14 years) than that of the patients remained in SR after cardiac surgery (58.7+11 years). Only 42.5 % patients in POSR had more than 60 years old comparative with 75.6% patients in POAF.

Several risk factors were analyzed in relation with occurrence of POAF in cardiac surgery. In univariate analysis, the only independent clinical predictors for POAF risk were found to be: age>60 years, male gender, ejection fraction <50%, increased pulmonary

hypertension, interstitial fibrosis, myocytolysis, nuclear abnormalities and pericardial adiposity (tab. II.2.2).

TABLE II.2.2. Risk factors in POAF vs POSR

	POSR-%	POAF-%	P_value
Age > 60 years	42.5	75.6	P < 0.0001
Male	13.63	51.35	P < 0.0001
EF < 50	1.51	75.67	P < 0.0001
LAD > 44 mm	50	56.75	P=0.64
PHT	1.51	18.91	P=0.0005
SHT	77.27	72.97	P=0.69
Fibrinogen	25.75	64.86	P=0.34
Hospital stay > 14 days	39.39	29.72	P=0.44
HT	31.81	98.9	P=0.0001
AT	56.06	40.54	P=0.19
IF	31.81	97.75	P=0.0001
Myocytolysis	38.84	91.89	P=0.0001
Abnormal nuclei	30.30	91.89	P=0.014
PA	27.27	75.67	P=0.0001
PH	27.27	33.33	P=0.74

Abreviations: ejection fraction-EF; left atrialdilation-LAD; pulmonary hypertension-PHT; systemic hypertension- SHT; hypertrophy-HT; atrophy-AT; interstitial fibrosis-IF; pericardial adiposity-PA; pericardial inflammatory infiltrate-PII;

The left atrial size, measured by echocardiography, was over normal range in all individuals, representing 56.75% in POAF and 50% in POSR patients. There was no correlation between postoperative AF and atrial dimensions.

On histopathological examination, mild to severe myocytolysis was detected in most of the specimens, 34 of 37 (91.89%) patients in POAF group and 25 of 66 (34.84%) patients in POSR group (p_0.0001; fig. II.2.1.a, b). CM hypertrophy was observed in 98.9% and 31.81% of the patients in POAF and RSPO group, respectively (p_0.0001). CM atrophy was detected in 40.54% of patients in POAF group and 56.06% in POSR patient group. High levels of lipofuscin were observed in 59% of the patients with POAF compared with 38.84% of the patients remained in SR. In POAF group, the percentage of the abnormal nuclei in each specimen was higher than POSR group (91.89% versus 45.45 %, *p* _ 0.014). CMs with contraction band necrosis (CBN) were rare findings in both groups (fig. II.2.1.c, d), being associated with ongoing interstitial fibrosis only in patients with POAF (fig. II.2.1.d).

Several histopathological abnormalities were encountered within atrial interstitium, in both groups, but only interstitial fibrosis (IF) was statistically significant. IF was detected in 97.75% patients in POAF group and 36.36% patients in POSR group (*p*_0.0001), respectively. No interstitial inflammatory infiltrate was seen.

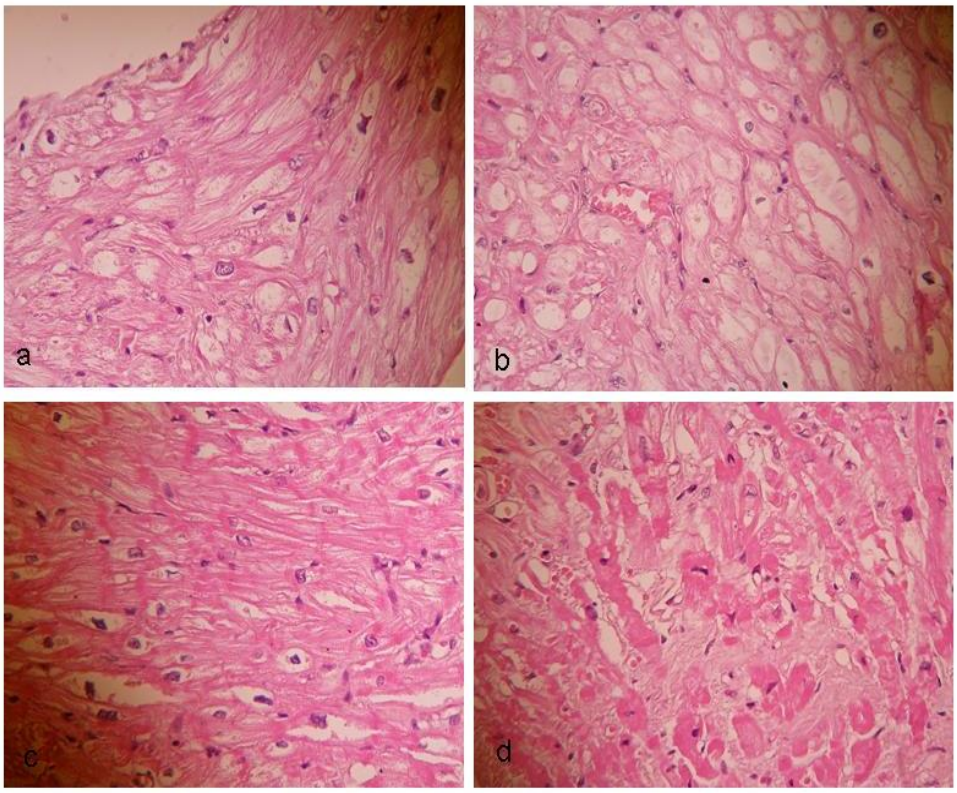

Figure II.2.1.a-myocytolysis in POSR; b- myocytolysis in POAF; c-CBN in POSR and, d- IF in POAF; (HE, 40x).

Pericardial adiposity (PA) registered a statistically significant difference between the two groups (75.67% in POAF; 27.27% in POSR; p_0.0001). Few pericardial inflammatory foci, related by localized pericardial fibrosis or slight extensions of pericardial adiposities were found.

II.2.4. Discussion

POAF still remains one of the most common causes of morbidity after cardiac surgery (Hogue et al, 2000). Although AF is a common postoperatory problem, the true incidence of POAF following cardiac surgery is unclear. Reported incidence ranges from 10%-65%. This range is wide because studies differ in baseline patient characteristics, type of surgery, methods of detection, and definitions of AF. POAF is approximately 30% after pure CABG surgery, 40% after valve replacements or repair, and increases to approximately 50% after combined procedures (Maisel et al, 2001).

Identifying the patients at risk for POAF after cardiac surgery would result in the reduction of both the incidence and undesired clinical consequences related to postoperative AF (Zaman et al, 2000). In addition to older age, many other risk factors have been identified, such as: male gender, decreased left-ventricular ejection fraction, left-atrial enlargement, valvular heart surgery, and chronic renal failure (Banach et al, 2006).

Age is the most powerful risk factor for development of POAF in most of the studies (Hogue et al, 2000). The frequency of this arrhythmia is increasing, most likely due to rising proportions of elderly patients undergoing cardiac surgery**.** In our study, the most probable

explanation for low incidence of POAF (35.92%) would be related to the relatively younger average age of our patients (61.77 years).

It was reported (Kitzman et al, 1990) that left atrial enlargement (LAE), fibrosis and atrophy in the atria, contribute to the susceptibility to develop POAF, but the link between these phenomena and AF after cardiac operation have not received enough scientific attention.

Some notes (Kitzman et al, 1990; Vaziri et al, 1994) refer to the fact that left atrial size is an important factor in AF development. These researchers noted that AF is rare (3%) when the left atrial dimension is below 44 mm, but is common (54%) when this dimension exceeds 44 mm. In other notes (Ausma et al, 2001), it shows that the atrial enlargement, due to structural remodeling, is a particularly important determinant of the occurrence of multiple-circuit reentry.

Interstitial fibrosis (IF) is considered a significant risk factor for POAF (Ausma et al, 2001). We found interstitial fibrosis in most specimens, generally, having higher values in POAF patients comparative with POSR patients (tab. II.2.2). As other scientists observation (Goette et al, 2002), we consider that in aged atrial myocardium, interstitial fibrosis could decrease conduction tissue. But, most of the histological changes are characteristic of ischemic myocardium, such as atrophy and fibrosis. The presence of cardiomyocyte atrophy was related by CM hypertrophy, as a compensatory lesion. So, atrophy (AT) and hypertrophy (HT) seem to be independent lesions in inducing background abnormality.

One of the most striking result of our study was the increased CM vacuolation in patients with POAF. CM vacuolation occurs either as a consequence of the normal aging process or in response to exposure to hypoxic stimuli in cardiac cells (Pirollo et al, 1985). Myocytolysis was appreciated (Ad et al, 2001) as the most important preoperative histopathological predictor for the development of POAF. We observed that both patient groups presented increased CM vacuolation as a possible arrhythmogenic substrate for development of POAF, but finally, only some of them had FAPO.

Another two histologic variables, appreciated as POAF predictors, were pericardial adiposity and inflammation (Ishii et al, 2005).

In our analysis we found a strong correlation between an extensive pericardial adiposity and POAF. It was suggested a relationship between pericardial adiposity and atrial fibrillation (Chekakie et al, 2010; Batal et al, 2010). It is noted that local effects of the proinflammatory cytokines released from the pericardial adipose tissue may be a potential AF mechanism (Corrado et al, 2008).

We revealed only few pericardial inflammatory foci, as possible AF trigger. It is considered that extracorporeal circulation contains enough systemic inflammatory mediators that may be, in part, responsible for the occurrence of POAF (Issac et al, 2007). Inflammation plays an important role in the pathogenesis of POAF, by altering atrial conduction, facilitating re-entry, and predisposing to the development of POAF (Ad et al, 2001).

Final remarks

In the present study, we examined the preoperative morphological status of the atria in correlation with clinical risk factors. Our results suggest that preoperative morphologic alterations like CM vacuolation and increased IF may constitute a pathologic substrate and predictive factor for POAF.

II.3. Immunohistochemical cellular interplay in postoperatory atrial fibrillation

II.3.1. Introduction

Atrial structural remodeling (ASR) depends on cardiomyocyte and interstitial myocardial injuries. ASR includes myolysis and hypertrophy of cardiomyocytes, a reversible program of fetal protein gene re-expression. These lesions cause a cascade of reactions that lead to atrial remodeling with structural, functional, electrical, and metabolic consequences (Akkaya et al, 2013*).*

Pathogenetically, atrial structural remodeling represents an adaptive response of cardiomyocytes, aimed to maintain homeostasis under the impact of external stress factors: tachycardia at a high depolarization rate together with volume and pressure overload. Specific stressors (ischemia, valvular disease, diastolic dysfunction, etc.) induce either functional adaptive reactions or maladaptive processes *(*Kourliouros et al, 2009*).*

The remodeling type and its degree correlated with the duration of exposure to stress factors: (a) a 30 minutes exposure to stress produced changes at the ionic level that may be reversible; (b) a week exposure to stress caused usually reversible damages at cellular level (hibernation); (c) exposure to stress for weeks or months determined apoptosis and fibrosis at cellular and extracellular matrix level (Gramley et al, 2009; Platonov et al, 2011).

In ASR, investigated during atrial fibrillation (AF), some authors reported reversible lesions, comprised of myocytolysis and hypertrophy (Corradi et al, 2008). Other authors described a process of cardiomyocyte dedifferentiation, in which dedifferentiation lesions included myolysis, hypertrophy, and reorganization of protein expression to fetal-like patterns, such as α-smooth muscle actin (α-SMA) and desmin (Driesen et al, 2009; Ouyang et al, 2009).

There are some similarities between cardiomyocyte remodeling in atrial fibrillation and hibernating myocardium. Hibernating Myocardium (HM) represents one entity describing the heart's adaptive responses to ischemia**.** In AF there is a process of cellular remodeling of hibernating myocardium, an evidence of myocyte dedifferentiation. AF and HM share similar cellular and molecular alterations. Review of the changes observed in atrial myocytes during prolonged AF and ventricular myocytes that develop a hibernating phenotype reveals several pathophysiologic traits that are shared by the two cardiac disease states. These characteristics support the hypothesis that AF and HM each elicit the activation of a conserved adaptive response to stress. This paradigm has important clinical implications in that an improved understanding of the mechanisms driving this remodeling may facilitate the identification of therapeutic targets to promote reversion of myocytes to a mature, healthy state and restore normal structure and function. This is particularly important given the relatively recent advent of new treatment strategies for each disease, that is, revascularization of HM and restoration of sinus rhythm in AF with cardioversion (Gramley et al, 2009). The cellular and molecular adaptations described above, and particularly their reversibility, may impact the recovery of function following these interventions, suggesting that adjunctive therapies aiming at accelerating reverse remodeling of the dedifferentiated myocyte phenotype may improve patient outcomes (Ouyang et al, 2009).

The following pathophysiologic traits are considered common features to atrial fibrillation and hibernating myocardium (Driesen et al, 2009): apoptosis-mediated myocyte loss; reactive cellular hypertrophy of remaining myocytes; reexpression of fetal genes/gene

isoforms (e.g., α-smooth muscle actin and myosin heavy chain); loss and/or redistribution of structural proteins (e.g., cardiotin, titin, and desmin); myolysis; sarcomere depletion; glycogen accumulation; alterations in size and/or shape of mitochondria (smaller mitochondria); downregulation of oxidative metabolism/fatty acid utilization; increased use of glycolytic metabolism; altered intracellular calcium handling; redox signaling and antioxidative response.

Objective

Until now, there are not enough studies on atrial structural remodeling able to reveal various types of adaptive atrial lesions. In this research, we focused the study on the cellular communication ongoing after postoperatory atrial fibrillation.

II.3.2. Material and methods

The study included 20 patients hospitalized for coronary surgery in 2012, 14 men and 6 women aged between 36 and 74 years.

Patients were monitored for diagnosing postoperative atrial fibrillation. The 20 coronary patients (10 patients with postoperative atrial fibrillation - POAF and 10 with postoperative sinus rhythm - POSR) were selected on clinical criteria: absence of transitory POAF, no concomitant hyperthyroidism, and no valvular disease. Tissue samples from the right atrial appendages of the POAF group of patients were compared with samples from patients who remained in POSR.

The paper was accomplished by combining histopathological, immunohistochemical (IHC), morphometric and statistical studies.

Histological processing was performed in accordance with current standard protocols for tissue harvesting and fixation. Microscopic assessment used an optical microscope: Olympus CX 41. By routine techniques (hematoxylin and eosin-HE) or special staining for collagen (Sirius Red-SR), we identified cellular and extracellular damages: myolysis and hypertrophy, and fibrosis. We also suspected dedifferentiation lesions that required IHC confirmation.

Immunohistochemistry was applied according to standard protocols for IHC staining techniques performed on paraffin-embedded tissues. IHC study allowed us to diagnose accurately in cardiomyocytes (CMs) dedifferentiated-type lesions, by assessing alpha-smooth muscle actin (α-SMA) and desmin markers.

Quantification of lesions was performed by morphometry using a color image analysis system: Quick Photo Micro 3.0. Data obtained were processed statistically and the results were expressed as mean values and percentages (small study group).

Myocytolysis means loss of myofilaments and appears as vacuolation of the cytoplasm. Morphometric quantification of myocytolysis related myocytolytic CM number to the total CMs number visualized on a high power field (HPF has a magnification of x400). We evaluated only myocytolytic CMs in which cytoplasmic vacuoles involved at least 25-30% of the cytosol. Myolysis was evaluated only in the cells containing the nucleus in the cross section plane.

Hypertrophy (HT) signifies 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. Hypertrophy was determined by measuring CM transverse diameter only in cells displaying the nucleus in the cross section plane.

Fibrosis is the result of increased myocardial interstitium by fibrous tissue and was revealed by Sirius red staining. Morphometric quantification of fibrosis was performed by relating the fibrous interstitial area (stained in red with SR dye) to the studied histological section area.

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.

Dedifferentiation, consisting of re-expression of fetal proteins, was detected by IHC analysis of CM proteins, α-SMA and desmin. Quantification was achieved by relating the total number of CMs displaying positive reaction for a certain protein to the total number of the CMs in the studied histological sections. CMs were considered dedifferentiated if they reexpressed markers specific to the fetal life and showed an attenuation of specific markers of adulthood.

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 CM number with IHC positive reaction referred to the total number of nucleated cells in the area taken into account.

II.3.3. Results

Qualitative structural atrial changes observed in both study groups were cellular and extracellular lesions: CM myocytolysis, CM hypertrophy, nuclear alterations in myocytolytic CMs and interstitial fibrosis. Structural changes were evaluated quantitatively by morphometry. In the quantitative study we quantified lesions on histological and IHC stained sections.

CM myocytolysis attained various degrees in the two studied groups. In POSR, myolysis interested about 1/5 cells (one fifth) out of the entire cell number (21.93%). In POAF, CM myolysis was slightly higher (28.61%). In POSR, we saw a uniform increase in CMs size without involvement of atrial architecture, while in POAF, various size CMs were present, with altered atrial architecture (fig. II.3.1.a, b).

CM hypertrophy was observed in both POAF and POSR patient groups, although we noted different proportions between the two study groups (in POSR = 8.57%; in POAF = 9.07%).

Interstitial fibrosis was identified in both groups, having various degrees, but a higher proportion in POAF (23.41%) than POSR (16.76%). In POAF patients, we found wide collagenous septa separating isolated large groups of CM cells, which affected electrical conduction, while in patients with POSR a high degree of fibrosis was observed only in elderly patients (fig. II.3.1.c, d).

IHC study allowed accurate diagnosis of dedifferentiation lesions suspected by us at histological examination made on usual or special stains. We studied immunohistochemically the dedifferentiated lesions by assessing cardiomyocyte proteins, α-SMA and desmin.

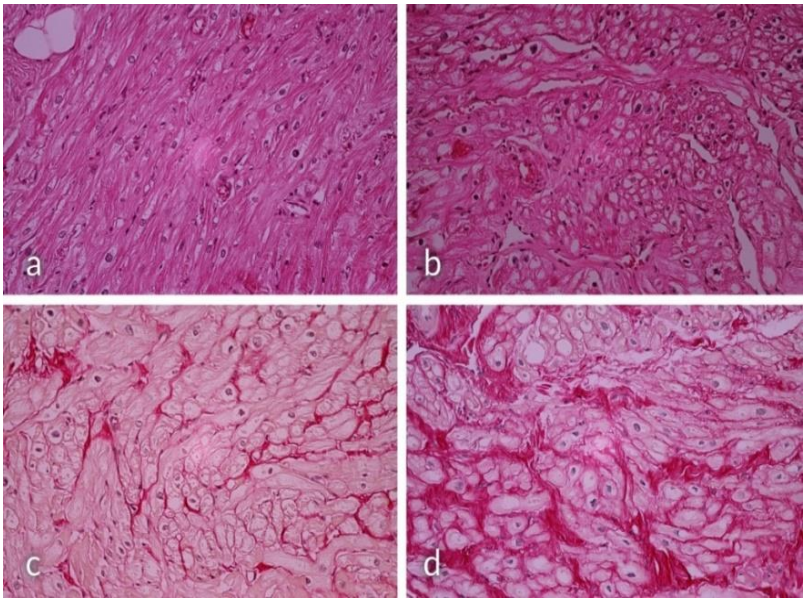

Figure II.3.1. (a) POSR, (b) POAF - myocytolysis (HE, x40);
(c) POSR, (d) POAF - interstitial fibrosis (SR, x40)

Normally, α-SMA is a contractile protein of fetal type, which is absent in adult type CMs. By dedifferentiation of CMs, a re-differentiation of this fetal-type protein (α-SMA) in adult-type protein (desmin) occurs. In adult atrial CMs, we found a positive reaction for α-SMA at the periphery of myolytic CMs. The degree of dedifferentiation was slightly higher in the POSR group (16.03%) than the POAF group (14.36%), suggesting more significant adaptive changes in patients with sinus rhythm (POSR) (fig. II.3.2.a, b).

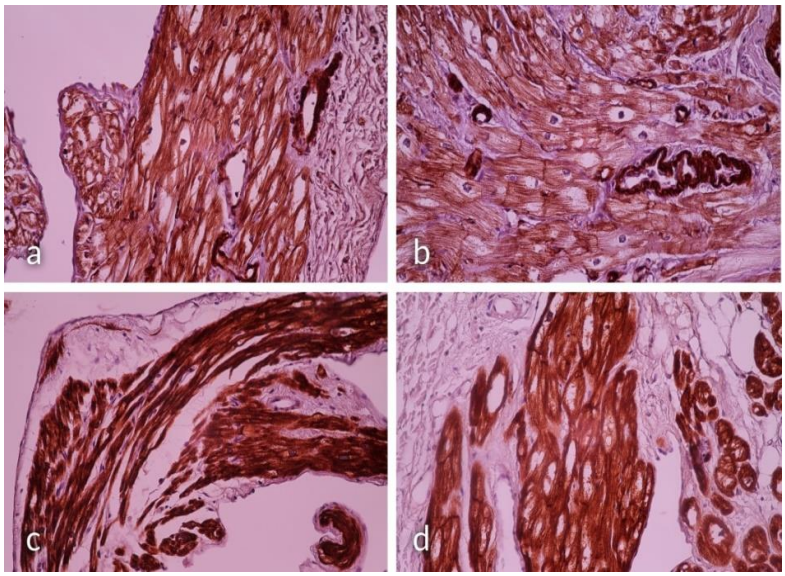

Figure II.3.2. (a) POSR, (b) POAF – atrial cardiomyocytes and vessels (α-SMA, x40);
(c) POSR, (d) POAF - atrial cardiomyocytes (Desmin, x40)

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.II.3.2.a, b).

Desmin is a protein characteristic of adult type phenotype of the cardiomyocytes. In the process of CM dedifferentiation we observed the reduction of desmin positive reaction expressed in adult type CMs in both groups. This aspect was revealed at the periphery of myolytic CMs and at the level of the intercalated disks. Positive reaction to desmin was somewhat lower in POAF (26.07%) than in POSR (29.41%), denoting greater loss of the CM contractile function in AF (fig. II.3.2.c, d).

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 than in POSR due to greater loss of CM contractile function in AF (fig. II.3.2.c, d).

II.3.4. Discussion

POAF is the most common cause of morbidity after cardiac surgery (Akkaya et al, 2013). Histological lesions were noted in most specimens, especially showing more advanced damages in POAF patients than in POSR patients (Platonov et al, 2011).

The study indicated that patients with coronary artery disease developed deep structural changes in atrial myocytes.

Morphometric data showed remarkable differences between patients with POSR and POAF such as the appearance of large vacuoles in CMs, suggesting the role of associated factors, including the patient's metabolic status, in AF development. In our series, patients' atrial myocytes predominantly displayed myolytic changes similar to those described by other authors (Driesen et al, 2009; Ouyang et al, 2009). Vacuolation was shown to occur during reversible myocardial damage and was suggested to be a predictor of vulnerability (Casaclang-Verzosa et al, 2008; Takeda et al, 2011).

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

Also hypertrophy, as an adaptive reaction, was twice as frequent in POAF patients as in the POSR ones. Usually, myocardial hypertrophy is a response of cardiac muscle to altered conditions of haemodynamic overload caused by a large number of physiological and pathological associated conditions (Selvetella et al, 2004).

Irrespective of age, atrial fibrosis increases susceptibility to developing POAF (Kourliouros et al, 2009). In our study, interstitial fibrosis was of 1.5 times more frequent in POAF than POSR patients. We consider fibrosis, representing a sign of ischemic myocardium, which could decrease the tissue conduction.

IHC-ly, 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 and delicate subendocardial smooth muscle bundles**,** as in other research paper (Takeda et al, 2010).

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 (He et al, 2010). In our study,

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.

Impaired cardiac atrial function in patients with coronary heart disease leads to changes in structural CM proteins. Such changes are described as adaptive reactions of dedifferentiation, indicative of fetal phenotype. Intra- and extracellular changes contribute to changes in the electrical circuitry rendering the atrium more vulnerable to the development of atrial fibrillation.

Final remarks

Our study detected a wide range of atrial structural changes, including dedifferentiation lesions. In fibrillating atria, the myolytic myocytes are in a dedifferentiation state similar to that of immature CMs, proving that dedifferentiation may be the best way for CMs to survive in case of prolonged exposure to adverse conditions.

II.4.The involvement of epicardial adiposity and inflammation in postoperatory atrial fibrilation – IHC and morphometrical study

II.4.1. Introduction

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 (Nattel et al, 2000). Interstitial inflammation and increased interstitial fibrosis has been observed having a role in the formation of local conduction blocks (Kostin et al, 2002).

Both systemic and local inflammation are involved in POAF pathogenesis

Systemic inflammation and oxidative stress

Surgical trauma, ischaemia from the initiation and prolonged use of cardiopulmonary bypass (CPB), and reperfusion lead to oxidative stress and production of pro-inflammatory molecules, resulting in endothelial and leucocyte activation, the release of NADPH oxidases, nitrous oxide production and reactive oxygen species generation (Zakkar et al, 2015; Ishii et al, 2005). Human studies have demonstrated an association between systemic inflammation and oxidative stress and the development of POAF (Kramer et al, 2015). This association is supported by a demonstrated decrease in POAF from anti-inflammatory prophylaxis using corticosteroids (Whitlock et al, 2008).

Local inflammation and oxidative stress

Pericardial disruption causes local inflammation around the heart and an increase in pericardial fluid volume. Postoperative pericardial fluid is highly oxidative and contains blood, haemoglobin and high levels of inflammatory markers that reflect leucocyte and platelet activation (Whitlock et al, 2008). The myocardium itself also produces pro-inflammatory molecules which contribute to local inflammation and directly alter cardiac function (Zakkar et al, 2015). Inflammation within the pericardial space results in cardiomyocyte apoptosis and altered electrical activity, which allows heterogenous action potentials and arrhythmias to form and propagate (Whitlock et al, 2009). Animal models have shown that the degree of atrial

inflammation directly corresponds to the incidence of POAF. Contact between inflammatory cells and cardiac tissue likely plays a role in the pathogenesis of POAF, although the exact mechanisms have not yet been elucidated (Ishii et al, 2005)

In addition to the interstitial inflammation, a large research effort has been dedicated to identify new biomarkers and therapeutic targets for arrhythmia. Recently, a relationship between the thickness of epicardial adipose tissue (EAT) and the incidence and severity of AF has been reported (Hatem et al, 2014). 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 (Sacks et al, 2007). 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 (Bertaso et al, 2013). The infiltration of adipocytes into the atrial myocardium could also disorganize the depolarization wave front favoring micro re-entry circuits and local conduction block (Hatem et al, 2014).

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.

Objective

This study aimed to determine the relationship between the epicardial inflammation, thickness of epicardial adipose tissue (EAT) and atrial fibrillation, as new biomarkers for postoperatory atrial fibrillation (POAF).

II.4.2. Material and methods

Patients

The study included 20 patients hospitalized for coronary surgery in 2012, 14 men and 6 women aged between 36 and 74 years.

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

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

Methods

The method consisted in combining histopathological, immunohistochemical (IHC) and morphometric analysis of the atrial surgical biopsies obtained from patients with POAF and POSR.

Histological examination was done in accordance with current standard protocols for paraffin-processed tissues by using (hematoxylin and eosin-HE) staining techniques. An

Olympus CX41 light microscope (Olympus, Tokyo, Japan) was used for histological evaluation and identification of RAA histological changes: epicardial inflammation and adiposity with consequent myocardial adipose tissue infiltration.

Epicardial adipose tissue area and inflammation assessement

Epicardial adipose tissue (EAT) measurement was assessed by reporting EAT area to the entire histological area on HPF.

Immunohistochemistry was applied according to standard protocols for paraffin-embedded tissues. Immunohistochemically we analyzed 10 histological sections at high magnification (x400 HPF) for each case.

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 IHC variables from patients with POSR and POAF. The results were expressed as average values and percentages (small study groups).

IHC examination focused on inflammation assessement by using CD3 for identifying T lymphocytes, and CD68 markers for macrophage evidentiation.

For assessment of inflammation, quantification was done by counting positive CD3 and CD68 cells in the field investigated at 400 x magnifications (HPF).

II.4.3. Results

From 20 selected patients aged from 36 to 74 years, with chronic myocardial ischemia, 10 were with POSR and 10 with POAF. Average age of patients with POAF was higher (64.7 years) than that of patients who remained in POSR after cardiac surgery (54.7 years).

In our study, we recorded an enlarged EAT and few epicardial inflammatory foci. The increased EAT area had a frequency of approximately 3 (2.96) times higher in POAF (68.03% of cases) than in POSR (22.94% of cases).

The IHC study allowed accurate diagnosis of epicardial inflammation (fig. II.4.1). The lymphocytic inflammatory foci (CD3+) within epicardial fat had higher values in POAF (75.6 %) in comparison with POSR patients (21.33%), as well. Epicardial macrophage inflammatory foci (fig. II.4.2) recorded a ratio of 4.31 between POAF (81.2%) and POSR (18.8%).

We also noted EAT myocardial consequences. Epicardial adiposity expansion into the myocardium (fig. II.4.3) determined the atrophy of some CMs, but without major differences between the 2 groups (FAPO, 51.5%; POSR, 40.3%). In POAF, CM atrophy may be a consequence of myocardial chronic ischemia including CM compression by adipose tissue infiltration.

Intramyocardial macrophages (Mfs) were identified IHC-ly within interstitial septa and perivascular areas. The proportion of macrophages varied slightly between the two groups of patients (FAPO, 17.4%; POSR, 13.3%). They may be resident interstitial cells.

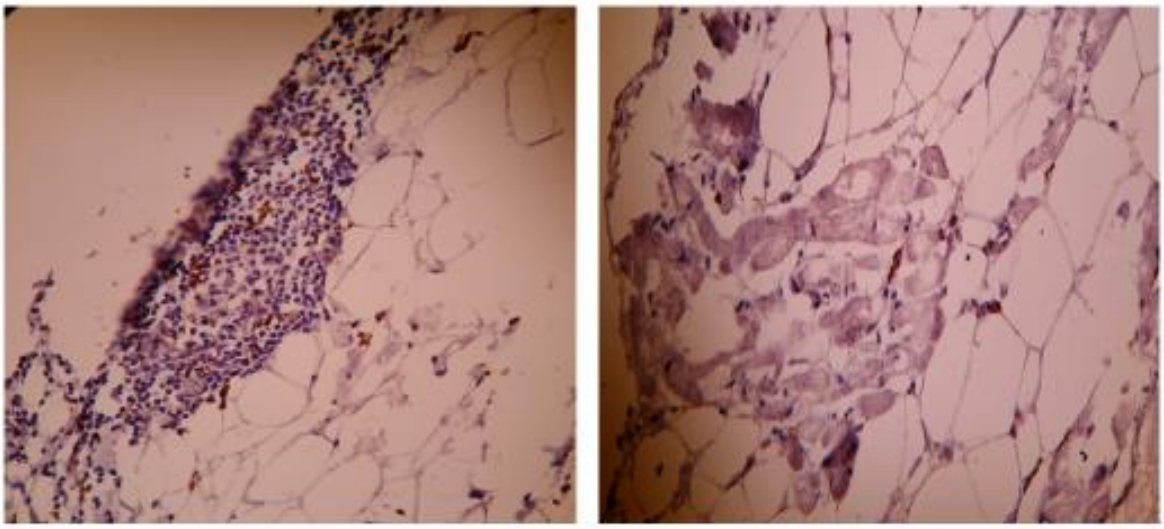

Figure II.4.1. Epicardial lymphocytes. Significant epicardial inflammatory focus related by EAT (a-POAF) and myocardial fatty infiltration (b-POSR); (CD3, x 20).

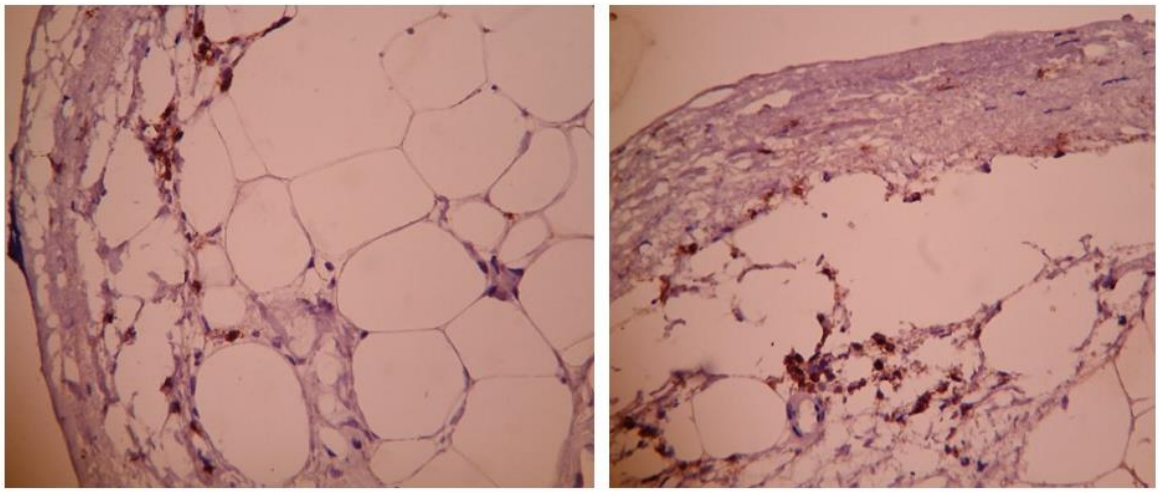

Figure II.4.2. Epicardial macrophages: (a) POSR, (b) POAF (CD68, x40).

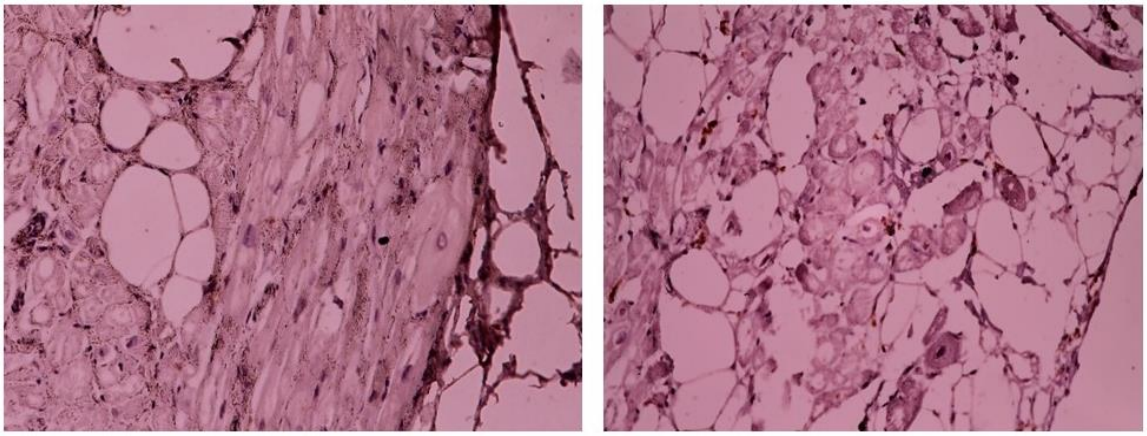

Figure II.4.3. Intramyocardial Macrophages: (a) POSR, (b) POAF (CD68, x40).

II.4.4. Discussion

The analysis associated histopathological, immunohistochemical, morphometric and statistical studies. Epicardial fat is the adipose tissue accumulated between the visceral pericardium and the myocardium.

The most common cause of morbidity after cardiac surgery still is POAF (Almassi et al, 1997). The study indicated that patients with POAF developed deep structural changes in atrial epi-myocardium. Other authors (Ad et al, 2001) noted structural changes in most specimens, generally showing more advanced damages in POAF patients than in POSR patients.

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 EAT and epicardial inflammation may constitute a pathological substrate for POAF.

In the last decade the interest in heart adiposity associated to consequent myocardial fat infiltration has renewed. It was noted that epicardial fat is a source of several proinflammatory cytokines (Tong et al, 2011).

However, it also secretes adiponectin with anti-inflammatory effects, as well (Hatem et al, 2014). 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 (Bogatu et al, 2016).

EAT expansion (Iozzo et al, 2016) 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.

Our data show that epicardial adiposity, measured by histo-morphometrical tools, seems to be associated with the presence and severity of AF. Measurements of epicardial fat in larger samples in association with AF are needed to confirm this finding.

Considering the myocardial inflammation, this reaction was reduced in POSR, but slightly raised in POAF, reflecting the tissue response to ischemic degenerative CM lesions, in the absence of a systemic inflammatory syndrome and a localized inflammatory infiltrate.

Inflammation plays an important role in POAF pathogenesis (Hirata et al, 2011), 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 POAF occurrence.

Although epicardial inflammation is absent in the normal epicardium, our results show the presence of focal epicardial mononuclear inflammatory infiltrates, especially in patients with POAF. CD3 marker was used for identifying T lymphocytes, which normally are absent within a healthy epicardium. CD68 is a useful marker for macrophage lineage. The precise amount of lymphocytes and macrophages within the diseased human heart is unknown (Kitzman et al, 1990). Other authors (Goette et al, 2002) appreciated that normal macrophage number varies significantly among patients (range 0±6 cells/high power field-HPF). We took in consideration lack of lymphocytes and presence of over 6 macrophages /HPF as abnormal atrial cell values.

Epicardial inflammation is suspected to be an independent risk factor, a possible mechanism in local or systemic inflammation (Hirata et al, 2011). Regarding EAT inflammation, our results suggest that inflammatory cell infiltration is enhanced in epicardial adipose tissue, mostly in POAF patients, probably reflecting local effects of the proinflammatory cytokines released from the epicardial adipose tissue. We suppose that inflammation in epicardial fat may influence the pathogenesis of AF in terms of trigger factor for arrhythmic heart.

Final remarks

Our results suggest that preoperative morphological alterations, like epicardial adiposity and inflammation may constitute a pathological substrate for POAF. Indeed, both epicardial adiposity and inflammation could be new cardiovascular markers in POAF and new therapeutical targets, as well.

II.5. Immunohistochemical evidence of growth hormone secretagogue receptor (GSH-R) and CD68 expression of atrial adipose tissue in obese POAF patients

II.5.1. Introduction

Obesity is a major cause of adipose tissue (AT) inflammation. It is a pro-inflammatory condition in which hypertrophied adipocytes and adipose tissue-resident immune cells (primarily macrophages) both contribute to a state of chronic low-grade systemic inflammation (Makki et al, 2013; Butcovan et al, 2017).

Ghrelin (GHRL), a gut peptide consisting of 28 amino acids, is the endogenous ligand of growth hormone secretagogue receptor 1a (GHS-R1a) (Kojima et al, 2005). Subsequent research has shown that ghrelin and its various receptors are ubiquitous in many peripheral organs including the stomach, intestine, pancreas, thyroid, adrenal gland, kidney, heart and blood vessels (Kojima and Kangawa, 2019). Moreover, they participate in the regulation of appetite, energy, bodyweight, metabolism of glucose and fat, as well as modulation of gastrointestinal, cardiovascular, pulmonary, immune functions, etc. (Kojima et al, 2005). Ghrelin may have cardiovascular protective effect, including lowering of blood pressure, regulation of atherosclerosis, and protection from ischemia/reperfusion injury as well as improving the prognosis of myocardial infarction and heart failure. In a previous paper we showed that ghrelin (GHRL), through its growth hormone secretagogue receptor (GHS-R) present on adipose tissue macrophages (ATMs), could modulate adipose tissue inflammation (Timofte et al, 2019). Some of these new functions of ghrelin may provide new potential therapeutic opportunities for ghrelin in cardiovascular medicine.

Postoperative atrial fibrillation (POAF) is a common complication after cardiac surgery in obese patients. Atrial fibrillation (AF**)** is the most frequent cardiac arrhythmia in clinical practice and is often associated with profound structural alterations of the atrial myocardium, including increased epicardial adiposity (Kostin S et al, 2002). Adipose tissue is a biologically active organ releasing adipokines. It is also a major source of cytokines (Hatem et al, 2014). Among others cardiac structural changes in obese POAF patients, epicardial adiposity and inflammation could be associated with increased cardiovascular risk (Butcovan et al, 2017).

There are no enough studies on the association between epicardial adipose tissue (EAT) area, GSH-R and EAT inflammation in POAF obese patients. Little is known about the relationship between ATMs and GSH-R in adipose tissue inflammation. Discovering a relationship between them and POAF in obese patients could open new research perspectives on the biology of the arrhythmogenic substrate.

Objective

The aim of our study was to investigate the association between epicardial adipose tissue (EAT) area, GSH-R and EAT inflammation in adipose tissue samples of human right atrial appendages (RAA) biopsies and developing postoperative atrial fibrillation (POAF) in obese patients by assessing immunohistochemical expression of CD68 and GHS-R in EAT.

II.5.2. Material and methods

Samples of UAD containing epicardial adipose tissues were obtained from 10 obese POAF patients, undergoing cardiac surgery for CABG and developing post surgery atrial fibrillation (POAF).

Histological examination and morphometry

Tissue samples were fixed in buffered formaldehyde and incorporated into paraffin blocks for the study of histology.

An Olympus CX41 light microscope (Olympus, Tokyo, Japan) was used for histological evaluation and identification of RAA cellular changes: enlargement of epicardial adipose tissue (EAT) and EAT inflammatory cell infiltration.

Morphometrical analysis was performed by using a color image analysis system: Quick Photo Micro 3.0.

We established two study groups, according to the presence or absence of EAT inflammation.

Cardiac adipocytes (CA) size wad done by assessing CA diameters on transversely sectioned adipocytes displaying the nucleus on cell contour. Morphometry was applied on 10 random high-power fields (HPF, x400) of epicardial adipose tissue (EAT). We calculated the mean adipocyte diameter (MAD) and the mean EAT area (contour EAT area). MAD results were expressed as mean values for each main group (μm). EAT area, for each main group, was expressed as mean values (μm2).

Immunohistochemistry and morphometry of CD 68 and GHS-R

An immunohistochemical method was used for assessing the GSH-R (GHSR Polyclonal Antibody, ThermoFisher Scientific, PA5-28752, 1/500) and CD68 (Dako, IS613) expression of the macrophages in epicardial adipose tissue samples from obese POAF patients.

Immunohistochemistry (IHC) and morphometric examination were applied to each of the 10 samples (5 samples for each main group). The positive immunoreactivity of macrophages at CD68 and GSHR (numerical value) was evaluated and the mean value was calculated.

The mean values for GSH-R and CD68 immunoreactivity were reported to the mean EAT area. We calculated the percentage of the inflammatory cells reported to the measured area. The results were expressed as mean values (number) and mean percentage values reported

to the adipose tissue area. The EAT inflammation was considered in samples with an increased number of macrophages (mean value >6)

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 21 Software. Numerical data are reported as means ± SD. Categorical data are reported as % number to EAT area.

II.5.3. Results

Histological examination of adipocyte size in epicardial adipose tissue (EAT)

EAT inflammatory cell infiltration was assessed, allowing us to compare the atrial structural changes between the two main groups of obese POAF patients (EAT with or without inflammation) (fig. II.5.1). EAT changes observed in both study groups consisted of increased EAT area, mainly by increasing of CA sizes.

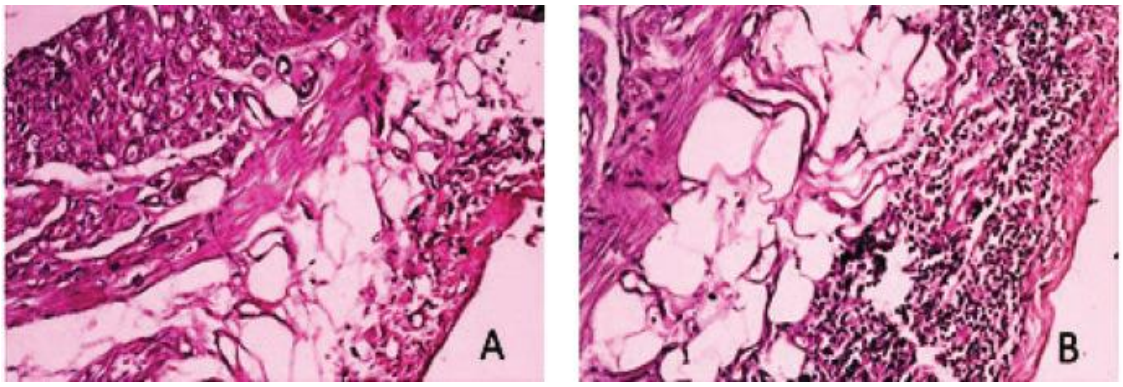

Figure II.5.1. Epicardial adipose tissue (EAT) (HE, x400).
A. EAT without inflammation; B. EAT with inflammation

TABLE II.5.1. Mean adipocyte diameter (MAD), EAT area and immunohistochemical markers of macrophages in peri-atrial EAT samples from obese POAF patients, with or without inflammation.

Peri-atrial EAT samples	POAF Patients (No)	MAD (µm)	EAT area (μm^2)	CD68 No/ μm^2	CD68 %	GSH-R No/ μm^2	GSH-R %
Without inflammation	5	79.6±38.6	155.8±27.9	4.8±1.3	2.8±0.8	6±1.8	3.5±1.5
With inflammation	5	84.2±45.6	172.1±21.3	10.6±3.7	6.8±2.2	9.4±4.1	6.1±3.6

EAT: epicardial tissue inflammation; MAD: mean adipocyte diameter; SD: standard deviation; The data are presented as mean (± standard deviations) values.

Histologically, the mean adipocyte diameter (MAD) of epicardial adipose tissue (EAT) was larger in EAT samples with inflammation than in EAT samples without inflammation (84.2 vs 79.6 µm) (table II.5.1).

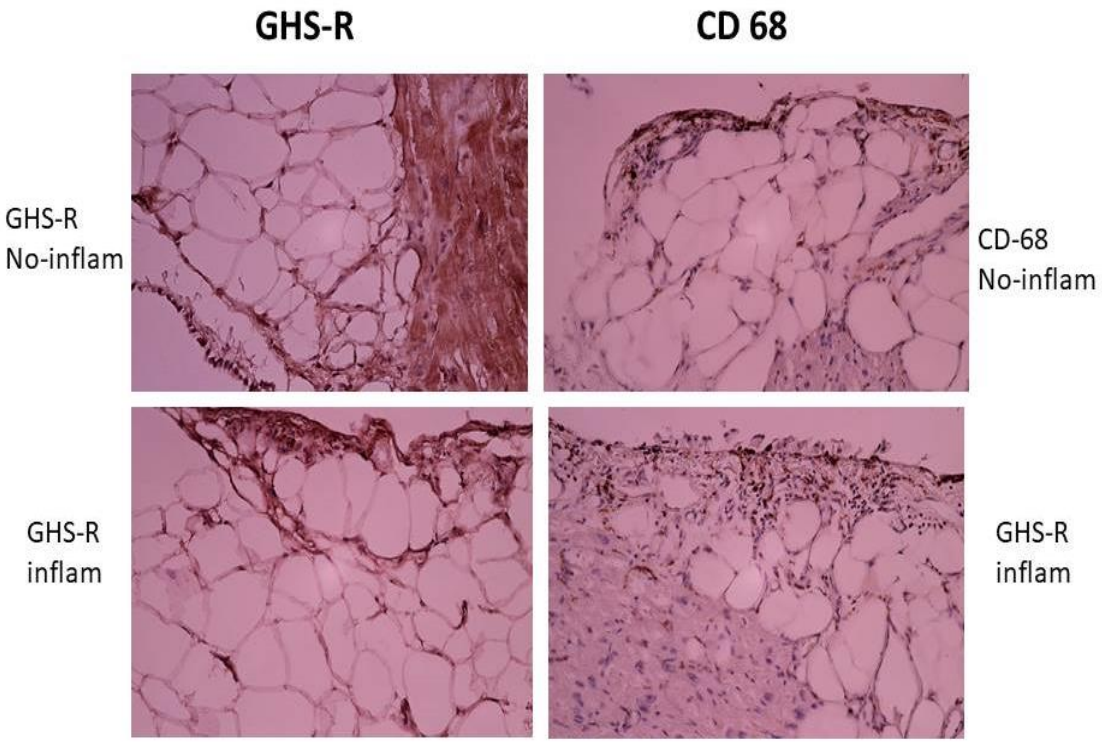

Figure II.5.2. GSH-R and CD68 expression of resident macrophages in peri-atrial epicardial adipose tissue (EAT) from obese patients. (GHS-R and CD68, 400x).

Immunohistochemistry of CD 68 and GHS-R

The expression of CD68 was lower in EAT without inflammation as compared to EAT with inflammation in adipose tissue samples (tab. II.5.1; fig. II.5.1). Similarly, the expression of GSH-R was lower in EAT samples without inflammation as compared to EAT with inflammation in adipose tissue samples (tab. II.5.1; fig. II.5.2).

II.5.4. Discussion

Our study suggested that obese POAF patients developed EAT structural changes. EAT area enlargement in POAF patients with EAT inflammation was the first observation (Kostin et al, 2002). The second important result of our study was the increase in CA size, especially in obese POAF patients whose specimens displayed inflammatory injuries; the same observation was made by other authors, too (Weisberg et al, 2003). In a previous study, we identified enlarged CAs as a histological predictive risk factor in atrial fibrillation (Butcovan et al, 2017).

Another most significant result of our study was the variable presence of EAT inflammatory cells, probably reflecting the local effects of proinflammatory cytokines released from epicardial adipose tissue. It is known that epicardial fat is a source of several proinflammatory cytokines (Weisberg et al, 2003). However, it secretes adiponectin with anti-inflammatory effects, as well (Lucas et al, 2009). Regulation of local pro-inflammatory and anti-inflammatory balance in peri-atrial adipose tissue, together with anti-oxidants use, might be an important therapeutic target in AFprevention (Bogatu et al, 2016).

Our study was focused on the relation between CD68 and GSH-R expression of ATMs in epicardial adipose tissues collected from obese POAF patients. We found increased CD68

immunohistochemical expression in ATs from obese patients. This finding was consistent with inflammatory infiltration of adipose tissue in the obese. The increased CD68 expression was compared with other adiposity research reports (Russo et al, 2018; Lumeng et al, 2007) showing increased number of macrophage cells.

It was noted that macrophages originating from adipose tissue represent one of the main forces in the development of chronic inflammation in obesity (Weisberg et al, 2003). Adipose tissue also contributes to inflammation, secreting various hormones and cytokines which play crucial roles in obesity-associated metabolic complications (Wisse, 2004).

There is little information about the GSH-R expression in human adipose tissues (Timofte et al, 2019). Our current data showed positive GSH-R immunohistochemical expression of AT (especially, ATMs) in obese patients. Our study showed a certain association between macrophage infiltration (CD68+) and increased GSH-R expression in adipose tissue. Some reports noticed that GHS-R1a ablation could promote an anti-inflammatory process under both diet-induced and age-associated adipose inflammation (Ma et al, 2013). It was noticed that GHS-R1a deletion activates anti-inflammatory macrophages, while increased GSH-R1a was associated with inflammatory macrophages (Sun et al, 2011).

Taken together these results could suggest that ATMs which express GSH-R may have a pro-inflammatory phenotype. The immunohistochemical analysis could be a useful test to assess the macrophage functions and to predict the future development of obesity-associated metabolic complications.

S*tudy limitations*

Our study has some limitations: First, the number of patients in the study was reduced. Increasing the number of patients studied would lead to obtaining a more accurate data analysis. Second, it is not clear whether local inflammation alone was the only factor in AF development. To clarify this issue, more studies are needed to be focused on both systemic and localized inflammation about EAT and AF persistence.

Final remarks

Our data suggested that the accumulation of CD68-macrophages of peri-atrial epicardial fat could be a trigger factor for arrhythmic heart in obese patients. The study also reported an increased immunohistochemical expression of GHS-R associated with increased macrophage infiltration of adipose tissue. These immunohistochemical markers could be related to insulin resistance and metabolic complications, which are cardiovascular risk factors of AF in obese cardiac patients.

CHAPTER III. HISTOPATHOLOGICAL EXAM VALUE IN CARDIOVASCULAR PATHOLOGY

III.1. State of the art

Histological exam is applied in both vascular (A) and cardiac pathology (B) as a significant diagnostic tool, either in common or rare, benign and malignant, myocardial conditions.

A. Histopathological analysis of the vessels

The most common arterial disease is atherosclerosis (ATS), where the pathologist interest is focused on vulnerable and unstable ATS plaque and assessing pre-existing early ATS lesions on conduit grafts prior of their use in coronary artery bypass (CABG).

Blood vessels are dynamic conduits, undergoing significant changes in response to the aging process and the development of vascular diseases.

Histologically, atherosclerotic changes were studied on endarterectomy specimens (from atherosclerosis cases who underwent atherectomy) and vascular surgery biopsies (arterial or vein conduits used as CABG grafts) using hematoxylin-eosin and trichrome stainings for demonstrating arterial wall changes and for assessing the disease stages (Escaned et al, 1993).

A1-Atherectomy, such as carotid endarterectomy (CEA), is applied in patients with severe carotid artery stenosis. The surgery is made under general anesthesia. A longitudinal incision is made along anterior border of the sternocleidomastoid muscle to expose carotid sheath. Vascular clumps are used to occlude common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA). CCA and ICA are longitudinally opened along the anterior vessel walls. The atheromatous plaque and nearby intima are carefully removed from the carotid bifurcation.

Carotid endarterectomy is performed using standard surgical techniques with minimal handling of the specimens. The plaques are removed en bloc, fixed in 10% buffered formalin, transected transversely in 5-mm specimens, and embedded in paraffin. Nowadays this study is made by using routine, special stainings and IHC methods for cellular identification and morphometry to quantify the degree of changes and for establishing the lesion subtypes according with various morphological classification scheme for lesions proposed by the American Heart Association (AHA) adapted to different arteries (Stary et al, 1994; Stary et al, 1995).

The presence of recent or organising thrombus, cholesterol clefts, and smooth muscle cell proliferation are assessed in atherectomy samples using standard histological techniques (Davies et al, 1993). The evidence of hemosiderin laden macrophages is considered to indicate old haemorrhage (Perls staining). A storiform pattern of smooth muscle cells in a connective tissue matrix background termed “accelerated progression pattern” was also evaluated on the hematoxylin eosin and thrychromic stained sections (Davies et al, 1993). Monoclonal antibodies were used to identify smooth muscle cells and macrophages within atherosclerotic plaque fragments. The presence of cellular components are assessed in a semiquantitative manner (1+ to 4+) by an experienced operator.

A2- Histological analysis of vascular conduits. The main etiology of coronary artery disease is atherosclerosis, a multifactorial chronic inflammatory process. Obstruction of blood flow is caused by plaques formed from fatty deposits in the intima of the blood vessel walls. The degree of obstruction of the vessel determines the treatment needed, either pharmacological or surgical (Greenwald, 2007). The most frequently occluded coronary arteries used are as follows: anterior interventricular artery (AIA), circumflex artery (CX), and right coronary artery (RCA) (Escaned J, 1993).

Coronary artery bypass grafting (CABG) using vascular conduits has become a standard procedure for coronary patients in many centers. Arteries or veins may be used in CABG as a graft to redirect blood to an area of the coronary artery, distal to the blockage (Escaned J, 1993). The vascular conduits are obtained during surgery for coronary revascularization with cardiopulmonary bypass (CBP). Intraoperative, the vessels, arteries and vein, are harvested by two experienced surgeons exclusively. Surgery is performed with cardiopulmonary bypass (CPB), mild hypothermia (32°C), and blood cardioplegic arrest.

The choice of the optimal revascularisation strategy in patients with multi-vessel coronary artery disease (MVCAD) has been a great challenge. The first issue is whether to use a venous or an arterial conduit (Van Son, 1990). While vein grafts act merely as conduits, arterial grafts have the ability to adapt to different demands of blood supply and show specific functional properties. The structure of the arteries differs in elastic and muscular composition, thus some are more reactive to vasoconstrictors than others. Due to vasoreactivity, a functional classification for arterial grafts into three subtypes was proposed (Da Costa, 1996): type I, somatic arteries; type II, splanchnic arteries; and type III, limb arteries. Due to the higher degree of smooth muscle cells over elastic fibres, type II and III arteries show higher contractility and are prone to spasm (Da Costa, 1996; Van Son, 1990). In general, the main advantage of arterial grafts is their superior long-term patency compared with saphenous vein grafts (SVGs) and, accordingly, arterial grafts are more indicated in younger patients (or in those who have a life expectancy of more than 10 years), which is beyond the benefit of SVGs (Hilal et al, 2013). Usually, the left internal mammary artery (LIMA) is grafted to the LAD in all patients. The second target vessel is always grafted with the radial artery (RA), in most cases the marginal branches of the circumflex artery.

Our research was focused on the assessement of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens that could cause atherothrombosis and to analyze the morphological characteristics of the conduits used in CABG (SV, IMA or ITA, RA) and to find the histological features (normal vessels are free of atherosclerosis) that could determine the behavior of the grafts and its permeability over time.

B. Histopathological analysis of endomyocardial biosy

The rare myocardial diseases represent another purpose of the pathologists that are involved in research studies using cardiac surgery or endomyocardial biopsies tools.

The Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology wrote a consensus document on when and how endomyocardial biopsy is of help for clinicians in the diagnosis and treatment of patients with heart failure, arrhythmias, and cardiac masses. Endomyocardial biopsy (EMB) is the gold standard for a

definitive diagnosis in disease entities like myocarditis, cardiac allograft rejection, infiltration/storage myocardial disorders, etc (Leone et al, 2012).

EMB technique

EMB involves percutaneous insertion into the heart of a ultrasound-directed sheath, which allows safe and rapid insertion of the bioptome and facilitates obtaining multiple specimens. Bioptic samples can be taken from the right ventricle, via the venous route through jugular, subclavian, or femoral veins, or from the left ventricle with transseptal puncture or by direct access through a peripheral artery, usually the femoral or brachial artery. Echocardiography allows accurate intracardiac insertion of the bioptome and may be useful in biopsy of cardiac masses (Veinot, 2002).

The modern view of diagnostic EMB requires the pathologist to have specific professional training (Thiene et al, 2010), use accurate specimen processing, use the traditional histological examination with histochemical, immunohistochemical (IHC), molecular, or ultrastructural tests (Thiene et al, 2010; Calabrese et al, 2002) and apply standardized diagnostic histopathologic criteria to minimize EMB reporting variability (Leone et al, 2012).

According to recommendations, endomyocardial biopsy should be performed in collaboration with cardiac pathology referral centers, where the whole arsenal of pathological investigation is available, including molecular techniques. Optimal use of the endomyocardial biopsy requires clinicopathologic correlations, too.

We applied the expert's recomandations in: (1) cardiac sarcoidosis (CS), (2) ARVC, and (3) cardiac malignancies.

1. Cardiac sarcoidosis

Recommendations

Sarcoidosis is a systemic granulomatous disease of unclear etiology. Assessment of cardiac involvement is important as it is the cause of death in half of the patients suffering of sarcoidosis**.** In some cases, an unexpected diagnosis of sarcoidosis is issued up biopsies collected from patients with ventricular tachycardia, suspected of ARVC or RCM. Sarcoidosis requires a differential diagnosis in the investigation of unexplained arrhythmias (Ladyjanskaia et al, 2010; Vasaiwala et al, 2009; Corrado et al, 2009).

EMB diagnostic potential

Due to the focal nature of the infiltrates, EMB has been reported to have poor sensitivity in systemic sarcoidosis with presumed cardiac involvement, being positive in 19%–25% of cases (Uemura et al, 1999; Ardehali et al, 2005). A positive biopsy, however, may reflect a more extensive disease and is associated with worse survival (Ardehali et al, 2005). More importantly, a positive biopsy guides therapeutic management of these patients with corticosteroids and potential prophylactic use of a defibrillator. To increase the sensitivity of the procedure, electrophysiologic (electroanatomic mapping) or image-guided biopsy procedures have been proposed.

Special technical aspects

Special stains for microorganisms are important to rule out infectious granulomatous diseases.

2. Arrhythmogenic Right Ventricle Cardiomyopathy (ARVC)

Recommendations

In selected cases having no clear-cut diagnosis with noninvasive and other invasive procedures, ARVC is a rare primary heart muscle disease with 1:2000–1:5000 prevalence in the general population. Genetic defects have been identified in genes mostly encoding for desmosomal proteins. The clinical presentation includes ventricular arrhythmias (Thiene, 1988), sudden death, particularly in the young and athletes, and also, less commonly, mechanical dysfunction (Nava et al, 2000). From the pathology viewpoint, ARVC is characterized by progressive atrophy of the ventricular myocardium with fibrofatty replacement. The septum is rarely involved. The clinical diagnosis is a major challenge since there is no single gold standard and it requires fulfilment of major and minor diagnostic criteria (scoring system) (Basso et al, 2009).

EMB diagnostic potential

If noninvasive and other invasive procedures do not allow a clear-cut diagnosis of ARVC, right ventricle endomyocardial biopsy (EMB) may serve as a complementary diagnostic tool. Moreover, in sporadic forms (non familial), EMB can be useful to exclude phenocopies (i.e., myocarditis, sarcoidosis, etc.). Histological examination, on hematoxylin–eosin and connective-tissue-stained slides, shows fibrous or fibrofatty replacement and myocardial atrophy, which are not specific. Fatty tissue alone is not considered diagnostic for ARVC since fat is normally found in the myocardium, particularly at the anterolateral apical right ventricular free wall. Histomorphometry evaluation of right ventricle EMB is mainly supported as a research tool to gather data. However, according to the updated diagnostic criteria, fibrous or fibrofatty replacement with <60% residual myocardium in at least one EMB sample is a major criterion, and 60%–75% residual myocardium is a minor criterion for ARVC (Basso et al, 2008).

Specific technical aspects

EMB samples from the septum may not be that helpful. If possible, consideration to biopsying the affected area, including the right ventricle free wall, may be considered. Diagnostic accuracy increases if the EMB sampling site is guided by imaging (MRI) or electrophysiologic (electroanatomic mapping) techniques.

Notes on IHC and molecular aspects

Immunofluorescence for plakoglobin and other cellular junction proteins is promising as it may decrease the need for free wall biopsy since it works on morphologically preserved myocardium and thus on septal bioptic samples. Paying attention to specific antibody dilutions and technique is important. However, this test needs validation before entering routine practice and cannot replace morphologic diagnosis at present (Asimaki et al, 2009).

3. Primary cardiac tumors

Recommendations

According to the World Health Organization (WHO) classification, primary cardiac tumors are categorized as benign tumors and tumor-like lesions, malignant tumors, and pericardial tumors (Fletcher, 2002). The most common primary cardiac neoplasm is the cardiac myxoma, a benign tumor that has no microscopic counterpart in other organs and tissues (Burke, 2004; Basso, 1997), whereas the most frequent malignant tumors are represented by

sarcomas, which have the same classification as soft tissue sarcomas (Burke, 2008). In autopsy series, the frequency of primary cardiac tumors was 1/1000, whereas cardiac metastases (mainly from lung, breast, or kidney, or from malignant melanoma) were far more prevalent (1/100) (Bussani et al, 2007).

Indications for EMB

For tumor assessment, EMB is indicated, although clinical diagnosis of cardiac masses is mainly done by echocardiography, computerized tomography, and/or MRI. Histology remains crucial for differentiating neoplastic from non-neoplastic masses and benign from malignant, and for subtyping the neoplasms. It therefore provides critical information for treatment and prognosis, which are largely dependent upon tumor histotype and biological behavior (Butany et al, 2005).

EMB is a valuable tool for preoperative diagnosis of intracardiac masses. It is mainly indicated for the investigation of right-sided masses showing an infiltrative or obstructive growth pattern and for the differential diagnosis of sarcomas, lymphomas, and metastatic tumors. Unresectable cardiac masses may benefit of EMB to help plan therapy or palliation (Basso, 1996).

Malignant primary tumors reported in the literature as diagnosed by EMB are angiosarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, etc.

Limits

False-negative results are possible due to sampling error but can be minimized by procurement of multiple specimens guided by echocardiography. Left-sided EMB is possible but sometimes avoided because of the potential for systemic embolism.

Technically, at least three to five formalin-fixed and paraffin embedded biopsy samples (2–3 mm^2 each) are advisable.

Immunohistochemically, the type of antibodies to be utilized depends upon tumor type. An initial useful suggested panel includes vimentin, factor-VIII-related antigen, CD31, CD34, epithelial membrane antigen, wide-spectrum cytokeratins, S-100 protein, smooth muscle actin, desmin, myogenin, HMB-45 molecule, CD45, CD20, and CD3 (Flipse, 1990).

Use of molecular biology techniques is recommended also. Fluorescence in situ hybridization and CRP tests are commonly used, at least in most referral centers, for the evaluation of sarcomas and lymphomas.

Rare myocardial Disease - Progress in cardiovascular research and clinical significance

The progress in cardiovascular research includes the study of rare diagnostic entities. Every year, based on basic and clinical research findings, new scientific statements or updates to clinical practice guidelines are published. Wide dissemination and implementation of them lead to improvements in our understanding of diseases and disorders and in clinical care. Progress is also observed in cardiovascular research concerning rare cardiovascular diseases and disorders (RCDD). However, due to the infrequency of these diseases and disorders and the relative paucity of reliable basic research and clinical evidence, collaboration between clinicians and scientists from many centres and various scientific backgrounds is needed. Such co-operation should also aim to gather evidence for better recognition of the pathogenesis of RCDD.

Rare cardiovascular diseases and disorders (RCDDs) constitute an important clinical problem, and their proper classification is crucial for expanding knowledge in the field of RCDDs.

The proposed classification provides an overview of RCDDs, which may also facilitate creation of databases and improved data gathering from various clinical centers. These disorders impact patient quality of life and /or mortality and insufficient knowledge about them may result in unfavorable clinical outcomes. Moreover, the study of them often requires multidisciplinary management.

The interest in this field lead to results published in 13 scientific papers (6 in journals indexed in international databases and 7 in journals indexed in Clarivate Analytics). The papers published in journals with impact factor (Clarivate Analytics) are presented below:

1. **Butcovan D**, Mocanu V, Baran D, Ciurescu D, Tinica G. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens. *Exp Ther Med* 2016; 11(5): 2028-2032. **IF=1.261**
2. Tinica G, Vartic CL, Mocanu V, Baran D, **Butcovan D**. Preoperative graft assessment in aortocoronary bypass surgery. *Exp Ther Med* 2016; 12(2): 804-808. **IF=1.261**
3. **Butcovan D**, Grigoriu C, Astarastoae V. Cardiac echinococcosis causing unexpected death. A case report. *Rom J Leg Med* 2010; 18(3): 189-192. **IF=0.301**
4. **Butcovan D**, Stoica L, Ungureanu C, Tinica G. Cardiac sarcoidosis - a possible cause of death. Case report. *Rom J Leg Med* 2010; 18(1): 13-16. **IF=0.301**
5. **Butcovan D**, Amalinei C, Grigoriu C. Arrhythmogenic right ventricular cardiomyopathy - cause of sudden death in young people. *Rom J Leg Med* 2011; 19(3): 189-194. **IF=0.398**
6. **Butcovan D**. Myocardial infarction in an individual with Wolff-Parkinson-White syndrome. *Rom J Leg Med* 2013; 21(1): 1-4. **IF=0.152**
7. **Butcovan D**. Cardiac sarcoma - a fatal disease: report of 2 cases; *Rom J Leg Med* 2012; 20(3): 173-176. **IF=0.208**

III.2. Insights in the Atherosclerosis Disease

III.2.1. Introduction

a. Atherosclerosis

Atherosclerosis is a progressive complex and multifactorial disease which affects medium and large arteries, of elastic and muscular type (Stary et al, 2008). Nowadays, according to Ross's theory confirmed by Stone, atherosclerosis is a chronic inflammatory disease (Stone et al, 2003) characterized by thickening of the arterial wall due to intimal accumulation of lipids and fibrous elements (Lusis et al, 2000).

Morphological classification

Atherosclerotic lesions are classified according to the American Heart Association (AHA) scheme (Stary et al, 1995), later adapted to coronary artery disease (Virmani et al, 2000), and more recently (Van Dijk et al, 2010) to specifically describe aortic atherosclerotic lesions (tab. III.2.1.a).

TABLE III.2.1.a. Atherosclerosis AHA classification, adapted by Virmani and van Dijk

AHA Classification-Stary	Van Dijk RA./Virmani R.
Early lesions	**Non-progressive intimal lesions**
Type I/Initial lesion	Adaptive intimal thickening
Type II/Fatty streak	Intimal xantoma
Type III /Intermediate lesion	
	Progressive atherosclerotic lesions
Advanced lesions	Pathologic intimal thickening
Type IV/Atheroma	Early fibroatheroma
Type IV-V/fibroatheroma	Late (advanced) fibroatheroma
Type VI/Complicated lesion	Thin-cap fibroatheroma
Type V-VII/Calcific lesion	Plaque rupture
	Healing rupture
	Fibrotic-calcified plaque

Lately, the Van Dijk/Virmani classification is used, followed by correspondence with AHA definition (Van Dijk, 2010; Virmani et al, 2000).

- **Non progressive intimal lesions**

Type I initial lesion1 / Non progressive intimal lesions / Adaptive intimal thickening

- *Adaptive intimal thickening / Type I initial lesion*

Microscopically, it is constituted of SMCs interspersed within a glycosaminoglycan and collagen rich matrix.

- *Intimal xanthoma/Type II lesion / Fatty streaks*

Type II lesions may have a maximum diameter < 1 mm, histologically composed of small accumulations of macrophages (foamy cells). A few inflammatory cells (mostly CD3-positive T-lymphocytes) and small glycosaminoglycan deposits may be associated (Stary HC, 2000).

- **Progressive atherosclerotic lesions**
  - *Pathological Intimal Thickening / Type III Intermediate lesion / Preatheroma*

Histology shows abundant collagen mixed with fibroblasts and a few scattered mononuclear inflammatory cells, and possible extracellular lipids (cholesterol crystals) (Stary et al, 2000). The elastic internal lamina may be altered.

- *Early Fibroatheroma / Type IV Lesion / Atheroma*

The plaque is formed by a central lipidic *core*, the atheroma and a peripheral area on the luminal side, composed of a fibrous cap. The central core is for the most part constituted by extracellular lipids, (cholesterol crystals and cholesterol esters) and necrotic debris, associated with overlying foamy macrophages and a variable amount of T-cells. The fibrous cap is constituted by collagen, elastic fibers, proteoglycans and SMCs.

- *Late (advanced) fibroatheroma / Type IV/V fibroatheroma*

Progression and coalescence of plaques produce the advanced fibroatheroma, which can affect large areas of the intimal surface and reach many centimeters in size in the late stage. The lesion becomes multi-layered as a consequence of progressive depositions of lipidic and fibrous components. Macrophages, T lymphocytes, neo-angiogenesis (new-formed capillaries, which are most commonly seen at the lateral site of the central core and fibrous cap),

haemorrhage or haemosiderin deposits (siderophages) are variably present (Khurana et al, 2004). The adjacent tunica media is often thinned, especially close to the largest plaques.

- *Thin cap fibroatheroma*

Thinning of the fibrous cap predisposes to plaque rupture. In the aorta, the fibrous cap thickness < 155 μm is described as a thin cap. Inflammatory infiltrates of the cap (through cytokines and metalloproteases release) and abundant extracellular lipid deposits (≥ 50% total plaque volume) concur in causing plaque rupture (Woollard et al, 2010).

- **Advanced lesions**
  - *Plaque rupture and healing rupture / Complicated lesions / Type VI*

Plaque Rupture is characterized by interruption of the fibrous cap, which may cause thrombus formation continuous with the underlying core. In these plaques, the necrotic core is usually abundant and the cap (most frequently thin) contains T-lymphocyte and macrophage inflammation. The rupture may also cause intraplaque haemorrhage, which rapidly increases the volume of the lesion (Virmani et al, 2000).

Plaque erosion, not described in Van Dijk's classification, is a different lesion corresponding to a superficial erosion of the endothelium without a real interruption of the fibrous cap.

Plaque rupture and erosion increase the risk of thromboembolic complications. (Virmani et al, 2000; Van Dijk et al, 2010).

- *Healing Plaque Rupture*

Plaque rupture may undergo spontaneous healing by vascular SMCs, which secrete extracellular glycosaminoglycan-rich matrix (Virmani et al, 2000; Van Dijk et al, 2010), variably associated to lipid, collagen, fibrin and platelet deposition.

- *Fibrocalcific Lesions*

Some plaques have thick, fibrous caps overlying extensive accumulations of calcium in the intima close to the media. Because the lipid-laden necrotic core, if present, is usually small, we refer to this category of lesion as fibrocalcific rather than atheroma. Of course, it is possible that the fibrocalcific lesion is the end stage of a process of atheromatous plaque rupture and/or erosion with healing and calcification (Kragel et al, 1989).

Our ATS study included (a) early ATS lesions, studied on vascular conduits used in CABG surgery, and (b) advanced ATS lesions, including vulnerable and unstable ones, studied on carotid endarterectomy specimens.

b. Comparative histopathology of vascular conduits and risk factors for development of intimal hyperplasia and atherosclerosis

The internal thoracic artery (ITA) graft is generally regarded as the standard conduit for CABG because of its excellent late patency and low prevalence of histopathologic changes. Favorable results from single and bilateral ITA grafting have led surgeons to pursue the use of other conduits for CABG, such as the radial artery (RA) and the saphenous vein (SV). In this study, we examined the comparative histopathology, morphometry and risk factors for the development of early ATS lesions, such as intimal hyperplasia and atherosclerosis, in the radial artery, the internal thoracic artery and saphenous vein.

Therefore, our research was focused on:

a. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens

- the evaluation of histological differences between plaques that are unstable and those that are vulnerable to instability.

b. Preoperative graft assessment in aortocoronary bypass surgery

- the assessing of pre-existing lesions in bypass grafts, that may contribute to a reduction in their viability, with early and late reduction of the graft patency

III.2.2. Materials and methods

a. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens

Patients

A total of 26 patients that underwent carotid artery endarterectomy to treat high-grade internal carotid artery stenosis at the "Prof. Dr. George I.M. Georgescu" Institute of Cardiovascular Diseases (Iasi, Romania) between January and December 2013 participated in the present study. Excised carotid ATS plaques were obtained from 20 male and 6 female patients, aged 35-80 years, who presented with transient ischemic attack symptoms upon diagnostic consultation.

Histological examination

Carotid endarterectomy was performed under anesthesia. The entire intimal carotid plaques (length, ~1 cm) were removed from the carotid arteries. The carotid artery atherosclerotic plaques were subsequently sectioned at 3-4 mm intervals and processed for histological examination. All sections were stained with hematoxylin and eosin, elastic Van Gieson, Masson's trichrome and movat pentachrome (all Bio Optica Milano SpA, Milan, Italy). Hematoxylin stains the nuclei and calcified material in cells blue, whereas eosin stains eosinophilic structures in various shades of red. Elastic Van Gieson is a histological stain for elastic and collagen fibers and Masson's trichrome and movat pentachrome stain muscle fibers, collagen and nuclei. Masson's trichome stain is used to detect connective tissue (green) and muscle tissue (red) characterized by fibrotic and degenerative changes; whereas movat pentachrome stain differentiates between the various ages and types of collagen and connective tissue matrix in the ATS plaques. Microscopic sections were analyzed with the observer blinded to the clinical status of the patients whose plaques were being examined.

Classification

ATS plaques were classified as vulnerable plaque (VP), stable plaque (SP) or unstable plaque (USP), as previously described (Stary et al, 1995). Lesions displaying a thin fibrous cap with infiltration of macrophages and a large necrotic core containing numerous cholesterol clefts were defined thin cap fibrroatheroma (TCFA), a type of VP. In carotid TCFAs, the area of the necrotic core is ≤ 3 mm^2, and the cap thickness of a vulnerable lesion is ≤ 165 μm. With regard to SPs, those with a large lipid-necrotic core containing extracellular lipid, cholesterol crystals and necrotic debris covered by a thick fibrous cap were considered to be FAs, whereas plaques with a small or absent lipid-laden necrotic core and a thick fibrous cap overlying extensive accumulations of calcium in the intima close to the media were considered FC plaques. In USP thrombotic plaques, thrombi occur as a consequence of one of the three

following events: PR, PE or, less frequently, CNs. PR was defined as an area of fibrous cap disruption in which the overlying thrombus was in continuity with the underlying necrotic core. PE was identified when a thrombus covers a fibrous cap with no defect. Typically, the endothelium is absent from the erosion site. CNs are lesions with fibrous cap disruption and thrombi associated with dense subendothelial nodules exhibiting calcification.

Histomorphometric analysis

Histological assessment was performed by an experienced pathologist using an optical microscope (CX41; Olympus Corporation, Tokyo, Japan). The measurements were visualized using color image analysis software (Quick Photo Micro 3.0, ProMicra, SRO, Prague, Czech Republic). Quantitative morphometry included measurement of the plaque, lipid necrotic core, inflammatory and calcified areas and fibrous cap thickness.

Risk factors assessment

The following potential risk factors (RFs) for atherosclerosis were also assessed: age, gender, diabetes mellitus (DM), arterial hypertension, history of cigarette smoking, cerebrovascular diseases and hyperlipidemia. All data on RFs data were obtained from the patients' files.

Statistical analysis

The association among the pathological characteristics of the three defined plaque types, as well as among plaque types and ATS RFs were evaluated. Data are expressed as mean values and percentages, calculated using Excel software (Microsoft Corporation, Redmond, WA, USA).

b. Preoperative graft assessment in CABG surgery-harvested conduit

Patients

A total of 26 patients who were undergoing surgical coronary revascularization at the "Prof. Dr. George I.M. Georgescu" Institute of Cardiovascular Diseases (Iasi, Romania) were enrolled in the present study between January 2013 and December 2013. Their ages ranged from 42-78 years (mean age, 60 years). The patients comprised 20 men (76.92%) and 6 women (23.08%).

Morphological and morphometric analysis of the grafts

A total of 54 distal segments of the ITA, RA and SV were evaluated. For histological examination, all sections were stained with hematoxylin and eosin (H&E), as well as with elastic Van Gieson **(**EVG) and Sirius red (SR) stains. Histological assessment was made with an optical microscope (Olympus CX41; Olympus Corporation, Tokyo, Japan). Measurements were conducted using a color image analysis system (Quick Photo Micro 3.0; Promicra, Prague, Czech Republic). Intima and media thickness and surface area were measured, in order to assess the intimal thickness index (ITI) and luminal narrowing.

Evaluation of the intimal thickness index and luminal narrowing

The degree of intimal thickening (by intimal hyperplasia and atherosclerosis) and luminal narrowing of the vascular conduits was evaluated by the determination of the intimal thickness index (ITI), which was calculated from the ratio of intimal and medial areas. The intima was defined as the distance from the lumen to the internal elastic lamella (IEL), in the area with the greatest intimal thickness. The media was considered as the distance from the internal elastic lamella to the adventitia, at the level of the greatest medial thickness. Two severity indices were calculated from the most severely diseased sections of the specimens

using the following formulae: i) ITI = intimal area/medial area; and ii) luminal narrowing (%) = intimal area/IEL area x 100. The ratio of the thickness of the intima to that of the media (R) was used as the index for arteriosclerosis, in accordance with the method of Kobayashi *et al* (Kobayashi et al, 1993). Atherosclerosis was graded on the basis of R as follows: Grade I, insignificant (R<0.1); grade II, mild (0.1≤R<1.0); grade III, moderate (1.0≤R<3.0); and grade IV, severe (R≥3.0).

Risk factor analysis

The prevalence of potential risk factors for atherosclerosis was assessed as follows: age, diabetes mellitus (DM), arterial hypertension (AHT), history of cigarette smoking, hyperlipidemia and obesity. The association between lesion severity and the number of atherosclerotic risk factors was investigated. Results are expressed as mean values or frequencies.

III.2.3. Results

a. Assessment of vulnerable and unstable carotid atherosclerotic plaques

Plaque types

A total of 26 carotid ATS plaques were histologically analyzed and classified as follows (tab. III.2.2.a): i) 6 cases of SP (23%), including 4 fibroatheroma (fig. III.2.1.a.-A) and 2 fibro-calcified plaques (fig. III.2.1.a.-B); ii) 14 cases of VP (54%), indicated by TCFA (fig. III.2.1.a.-C); and iii) 6 USPs (23%), including 2 cases each of PE (fig. III.2.2.a.-A), PR (fig. III.2.2.a.-B) and CNs (fig. III.2.2.a.-C).

Histomorphometric analysis

Morphometric analysis was performed to evaluate the fibrous cap thickness and necrotic core size, in addition to the inflammatory and calcified plaque areas of the ATS plaques (tab. III.2.3.a).

USPs exhibited the smallest fibrous cap thickness and a large lipid core area in the total plaque area, and exhibited inflammatory infiltrate. VPs were found to have a fibrous cap thickness of <164 μm and lipid core areas of varying sizes, and showed considerable inflammatory infiltrate. Plaque instability was found to be highly associated with fibrous cap thickness, lipid core area and plaque size. The incidence of the various RFs is summarized in table III.2.4.a.

TABLE III.2.2a. Carotid atherosclerotic plaques.

Plaque type	Patients (n=26)	
	n	%
Stable	6	23
Fibroatheroma	4	-
Fibro-calcified	2	-
Vulnerable	14	54
Thin-cap fibroatheroma	14	-
Unstable	6	23
Plaque erosion	2	-
Plaque rupture	2	-
Calcified nodules	2	-

TABLE III.2.3.a. Carotid atherosclerotic plaques measurements.

Plaque type	Fibrous cap, μm	Necrotic core, %	Inflammatory infiltrate, %	Calcification, %
FA	351 (173-654)	56.99 (32.24-87.19)	minimum	-
FC	270 (170-371)	46.86 (37-56.72)	-	6.23 (8.11-4.36)
TCFA	21.91 (5-44)	25.90 (3.04-60.22)	8.41 (0-25.87)	-
PR	11.66 (6-20)	22.03 (2.64-32.50)	3.04 (2.64-32.50)	-
PE	13.13 (10-16)	70.29 (51.97-87.74)	6.69 (5-8.35)	17.85 (14.11-21.46)
CN	20.33 (11-35)	-	minimum	136.599
FA, fibroatheroma; FC, fibro-calcified plaque; TCFA, thin-cap fibroatheroma; PR, plaque rupture; PE, plaque erosion; CN, calcified nodule.				

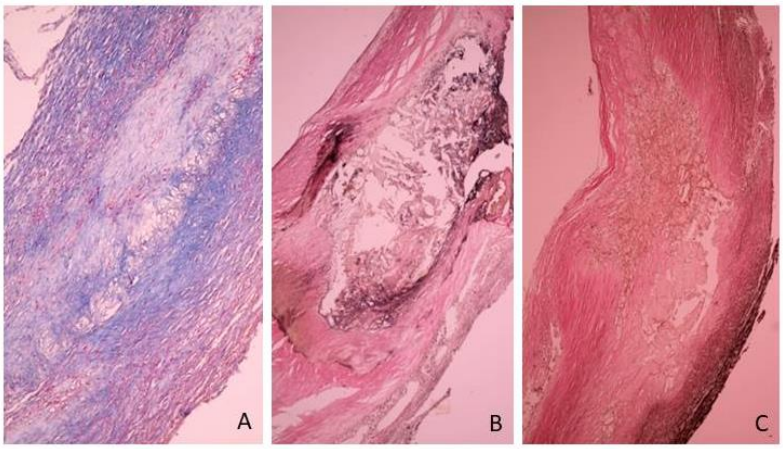

Figure III.2.1.a. (A) Fibroatheromatous plaque (stain, Masson's trichrome; magnification, x10); (B) Fibro-calcified plaque (stain, elastic Van Gieson; magnification, x10); (C) Thin-cap fibroatheroma (stain, elastic Van Gieson; magnification, x10).

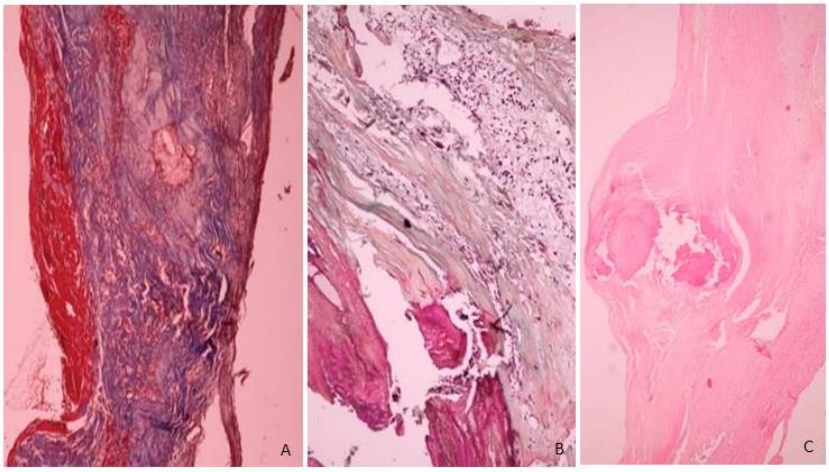

Figure III.2.2.a. (A) Erosion plaque (stain, Masson's trichrome; magnification, x10); (B) Ruptured plaque (stain, movat pentachrome; magnification, x10); (C) Calcified nodule (stain, hematoxylin and eosin; magnification, x10).

TABLE III.2.4.a. Incidence rates of risk factors in relation to ATS lesions.

ATS lesion	Risk factors (n)					
	Age >50 years	Smoking	AHT	HL	DM	CVD
SP	2	1	5	2	1	0
VP	10	4	14	8	4	10
USP	5	2	6	3	4	5
Total pts No (%)	17 (65.38)	7 (26.92)	25 (96.15)	13 (50.00)	9 (34.61)	15 (57.69)
ATS, atherosclerosis; AHT, arterial hypertension; HL, hyperlipidemia; DM, diabetes mellitus; CVD, cardiovascular diseases; SP, stable plaques; VP, vulnerable plaques; USP, unstable plaques.						

TABLE III.2.5.a. Incidence of the cumulative cardiovascular risk factors in each patient

ATS lesion	Cardiovascular risk factors (n)					
	6	5	4	3	2	1
SP	0	0	2	0	0	0
VP	3	4	10	1	0	0
USP	2	1	3	0	0	0
Total pts No (%)	5 (19.23)	5 (19.23)	15 (57.69)	1 (3.84)	0 (0.00)	0 (0.00)
ATS, atherosclerosis; SP, stable plaques; VP, vulnerable plaques; USP, unstable plaques.						

In carotid TCFAs, the area of the necrotic core is ≤3 mm_2, and the cap thickness of a vulnerable lesion is ≤165 μm.

The age of the patients, as well as the prevalence of the RFs for ATS differed between the SP and USP groups (data not shown). Arterial hypertension was found to be coexistent with the development of ATS plaques in 96.15% of all cases. Furthermore, age appeared to be an associated factor in ATS development. Hyperlipidemia was detected in 50% of all cases. DM was present in 34.61% of cases and was one of the investigated RFs involved in ATS progression in the vessels. Smoking was involved in 26.92% of all cases, suggesting that it is an important risk factor in early and late thrombosis. Involvement of other peripheral vessels in ATS process was identified in 57.69% of all cases.

Risk factors

The age of the patients and the prevalence of the RFs for ATS in the SP group differed from the USP group: In order of decreasing frequency, the RFs were higher in VPs compared with the USPs and SPs (data not shown). Table III.2.5.a shows the cumulative RFs in each patient.

Taking into consideration the number of the RFs involved in the pathogenesis of ATS in each patient, the following frequencies were observed (Tab. III.2.5.a): one patient (3.84%) had three RFs; 15 patients (57.69%) had 4 RFs; 5 patients (19.23%) had 5 RFs; and 5 patients (19.23%) had 6 RFs. No patient was found to have 1 or 2 RFs. The most frequent cumulative risk factors were identified in patients with VPs, followed by patients with USPs.

b. Preoperative graft assessment

Patient characteristics

All analyzed patients presented signs of unstable angina pectoris at hospital admission. Table III.2.1.b shows the vessels used for myocardial revascularization according to patient gender.

TABLE III.2.1.b. Vascular conduits according to gender.

Gender	ITA	RA	SV	Total
Male	20	10	18	48
Female	4	2	0	6
Total	24	12	18	54
ITA, internal thoracic artery; RA, radial artery; SV, saphenous vein.				

Morphological analysis

The results of the histological investigation (tab. III.2.2.b) indicated that morphological changes were present with high incidence in the walls of the fresh 'normal' vessels (ITAs, RAs and SVs) prior to their use as aortocoronary conduits. The identification of the presence of preoperatory vessel lesions is very important in the viability assessment of the graft conduits and for long-term assessment. According to their descending order of frequency, the graft lesions were represented by intimal thickening or hyperplasia (20 cases), medial fibrosis (17 cases) and fatty streaks (2 cases).

Morphological examination indicated that the ITA lesions consisted mainly of intimal hyperplasia associated with intimal thickening (fig. III.2.1.a-A), medial fibrosis (including 1 case with medial dissection) and fatty streaks (fig. III.2.2.a-B). The RA lesions consisted of intimal thickening (fig. III.2.1.b-A) and medial fibrosis, in equal proportions. SV lesions consisted mainly of intimal hyperplasia, which was rarely severe enough to narrow the lumen significantly, and medial fibrosis (fig. III.2.2.b-B).

Morphometrical analysis

In the morphometric analysis, the ITI of the vessel conduit was calculated as a measure of the degree of preoperative luminal narrowing dependent on intimal thickness (tab. III.2.3.b). The mean ITI values for the vessel conduits were 0.37 for the SVs, 0.95 for the RAs, and 1.66 for the ITAs. No patient had >50% conduit stenosis.

TABLE III.2.2.b. Lesion types in the different types of vessel conduit.

Vessel type	Normal	Lesion			
		Intimal hyperplasia	Atherosclerosis	Medial fibrosis	Total
ITA	7	10	2	5	24
RA	3	4	0	5	12
SV	5	6	0	7	18
Total	15	20	2	17	54
ITA, internal thoracic artery; RA, radial artery; SV, saphenous vein.					

TABLE III.2.3.b. ITI assessment and vessel conduit measurements

Atherosclerosis grade (R)	Degree of intimal thickening	Luminal narrowing	ITI (mean)			
			RA	SV	ITA	Total cases
Grade 0	Normal		0.00	0.00	0.00	0.00
Grade I (<0.1)	Insignificant	Minimal	0.00	0.00	0.00	0.00
Grade II (0.1-1.0)	Mild	<25% 25-50%	0.95	0.37	0.00	16.00
Grade III (1.0-3.0)	Moderate	>50%	0.00	0.00	1.66	10.00
Grade IV (>3.0)	Severe		0.00	0.00	0.00	0.00
ITI, intimal thickening index; R, ratio of intimal thickness to medial thickness.						

TABLE III.2.4.b. Incidence rates of selected risk factors associated with vessel conduits

Risk factor	Patients, n (%)
Age >60 years	16 (61.54)
Smoking	9 (34.62)
Arterial hypertension	17 (65.38)
Hyperlipidemia	17 (65.38)
Diabetes mellitus	10 (38.46)
Obesity	4 (15.38)

Risk factor analysis

Table III.2.4.b.shows the incidence rates of selected cardiovascular risk factors associated with vessel conduits. All patients showed risk factors for atherosclerosis, such as age >60 years, arterial hypertension, smoking, DM, obesity and hyperlipidemia. Arterial hypertension was found in 65.38% of all cases, having an impact on the development of atherosclerotic plaques and fibrointimal hyperplasia. Hyperlipidemia was also present in 65.38% of all cases. DM was present in 38.46% of cases; DM is associated with the progression of atherosclerosis in the native vessels and the functional impairment of veins where the level of prostacyclin production is reduced. Smoking was a factor present in 34.62% of all cases; it is an important risk factor for the early and late thrombosis of venous grafts. Obesity was less frequently involved, and was present in only 15.38% of all cases in the study group.

The association between the cumulative number of risk factors and degree of conduit stenosis is shown in Table III.2.5.b. Vascular conduit stenosis of <25% (ITI range, 0.18-0.95) was found in 16 patients and 88% of them (14/16) had three or fewer cumulative risk factors. The degree of narrowing was 25-50% of the vascular conduit (ITI range, 1.02-1.67) in 10 patients and 70% of them (7/10) had more than three cumulative risk factors.

TABLE III.2.5.b.Association of the degree of conduct stenosis with the number of CV risk factors.

ITI	Degree of narrowing	Number of risk factors							
		7	6	5	4	3	2	1	Total
Grade IV	>50%	-	-	-	-	-	-	-	-
Grade III	25-50%	1	2	2	2	2	1	-	10
Grade II	<25%	-	-	1	1	6	5	3	16
Grade I	Insignificant	-	-	-	-	-	-	-	-
Total		1	2	3	3	8	6	3	26
ITI, intimal thickness index.									

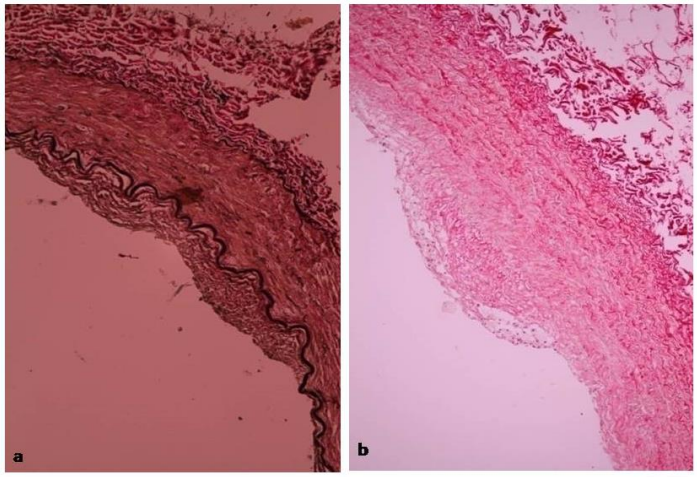

Figure III.2.1.b. Histological analysis of the internal thoracic artery, showing thickening of the intima. (A) Intimal hyperplasia (elastic Van Gieson staining; magnification, x200) and (B) fatty streaks (hematoxylin and eosin staining; magnification, x200).

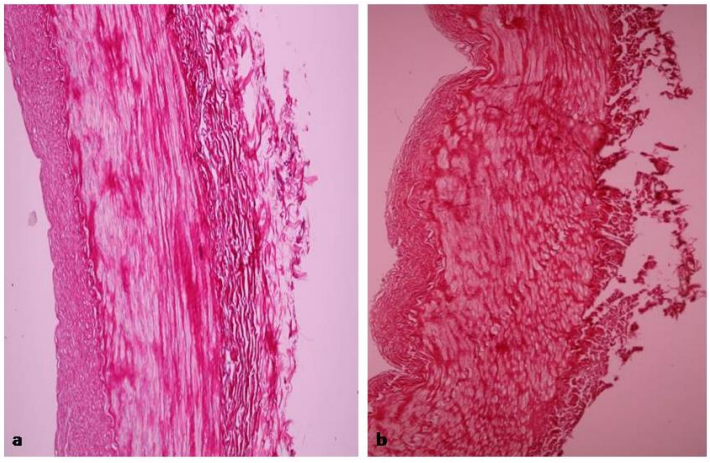

Figure III.2.2.b. Histological analysis of the (A) radial artery, showing thickening of the intima (Sirius red staining; magnification, x200) and (B) saphenous vein, showing thickening of the intima and media (Sirius red staining, magnification, x200).

III.2.4. Discussion

a. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens

Atherosclerosis is a major cause of morbidity and mortality worldwide, and despite the advances in the understanding of its pathogenesis, the factors that determine atheromatous plaque instability remain largely unknown. The prediction of plaque vulnerability to rupture and subsequent thrombosis would be useful in the development of diagnostic and therapeutic approaches (Moreno, 2010). By correlating histomorphological examinations and imaging results, the present study aimed to develop improved criteria for the diagnostic processing of ATS plaques.

Cap thickness has been identified as a crucial characteristic for the distinction between TCFAs and FAs, as TCFAs are known to have a thinner cap (Furie et al, 2011; Virmani et al, 2000). However, only 1 case of plaque rupture (2.66%) and 3 cases of TCFAs (12.5%) had a cap thickness >15 μm. It was suggested that monitoring cap thickness may be an option for predicting future behavior of an ATS lesion (Fishbein, 2010).

Further separation of plaque ruptures and TCFAs from FAs is indicated by the simultaneous presence of plaque inflammation and necrotic core area (Versteylen, 2013). Regarding FAs, definitory issues were cap thickness ≥165 μm and necrotic core area ≥3.5 mm^2 (Furie et al, 2011).

Excluding fibrous cap thickness from the analysis, plaque inflammation allowed for the separation of plaque rupture and TCFAs from FAs in a proportion of ≥3 times greater in VPs and RPs than in FAs (Fishbein, 2010). A discriminatory level of inflammation was found consisting in >0.2 mm^2 macrophage area/ microscopic HPF (Hirayama et al, 2009).

In a previous study, the mean macrophage area that infiltrated the fibrous cap and the shoulder of the plaque area was significantly greater in non-calcified plaque sections as compared with calcified sections (Hirayama et al, 2009). Although the present study had certain limitations, including the low sample size and lack of statistical analysis, the results indicated that a larger macrophage-rich area was present in the fibrous cap and the shoulder region of noncalcified plaques. Furthermore, noncalcified carotid plaques commonly exhibit a higher degree of fibrous cap inflammation, a key process in fibrous cap disruption.

Calcification in ATS lesions is relatively common and has been implicated as a risk factor for increased cardiovascular morbidity and mortality (Brajovic et al, 2009; Kim et al, 2009), while other authors demonstrated that carotid ATS plaque calcification is a structural marker for carotid plaque stability (Bayturan et al, 2009; Hashimoto et al, 2009).

In the present study, 14 plaques were defined as TCFAs. These plaques were not ruptured but were considered of high risk; thus, detection of such plaques is the key aim of diagnosis.

PRs and fissures usually occur in the fibrous cap and shoulder regions. Rupture of the ATS plaques is responsible for the majority of acute coronary events, and such lesions have been shown to exhibit distinct histopathological features (Mortaz et al, 2009; Finn et al, 2009). The present results indicated that fibrous cap inflammation and susceptibility to disruption are more likely to occur in noncalcified compared with calcified plaques. We speculate that the quantitative assessment of carotid plaque calcification using imaging modalities may help

identify patients with asymptomatic vulnerable carotid plaques who are at risk of cerebrovascular ischemic events and would benefit from carotid interventions.

Intimal thickness and lipid core area have been associated with RFs for coronary ATS disease (Lloyd-Jones et al, 2010), and may be valid markers of early carotid atherosclerosis from pathology. In the present study, the prevalence of hypertension, hyperlipidemia, and current history of smoking was higher in symptomatic than compared with asymptomatic cases. Therefore, the identification of RFs for carotid atherosclerosis among patients with coronary heart disease may provide an evidential basis for prevention.

Currently, among the most promising fields in the study of atherosclerosis is the development of imaging techniques that facilitate gross, microscopic and molecular characterization of *in vivo* ATS plaques (2). Modern visualization techniques are able to demonstrate the presence of inflammation, macrophage infiltration, angiogenesis, apoptosis, and other cellular and molecular features of plaques that may be involved in plaque destabilization and subsequent clinical events even in living patients (Motoyama et al, 2009). In addition, a recent computed tomography coronary angiography study (Versteylen, 2013) demonstrated that large plaques with calcified micronodules are the most prone to rupture. Such imaging techniques may facilitate the more specific characterization of ATS plaques and the identification of characteristics that are associated with and directly responsible for plaque rupture. All these approaches; however, should only complement histopathological investigations, which more specifically confirm the identity of lesions.

Atheromatous plaques may become unstable due to increases in size, increased intra and extracellular lipid accumulation, as well as intraplaque hemorrhage (Van der Wal and Becker, 1999). Based on these results, diagnostic modalities that detect plaque size, and hemorrhage, and/or lipid content are most likely to be useful in the prediction of unstable plaques. Furthermore, atheromatous plaques may become vulnerable due to fibrous cap thinning, which is associated with cap inflammation, a key process in fibrous cap disruption (Kolodgie et al, 2004; Virmani et al, 2005). Therefore, diagnostic methods that detect plaque size and hemorrhage, lipid content and the thinnest fibrous cap can prove considerably useful in determining USPs and VPs.

The most common RFs that were found to be associated with stable, vulnerable and unstable ATS plaques were age and hypertension. Hypertension in atherosclerosis indicates disease progression and may become life-threatening (Van der Wal and Becker, 1999); however, associations between specific risk factors and the composition of plaque are yet to be elucidated.

Final remarks

The vulnerable plaque concept, describing atherosclerotic plaques causing acute clinical events, has led to advances in our understanding of pathogenesis of atherosclerosis. The vulnerable plaque is a valid concept in principle, holding great promise for future research, pointing the need for an integrative approach to plaque assessment.

b. Preoperative graft assessment

Three types of vascular lesions were identified in the grafts, namely intimal hyperplasia, atherosclerosis and medial fibrosis.

Mild intimal hyperplasia was observed in the majority of the graft segments taken from the patients undergoing CABG. Intimal hyperplasia occurred more frequently in ITA grafts (10/24 cases, 41.67%) than in SV grafts (6/18 cases, 33.33%) and RA grafts (4/12 cases, 33.33%). Intimal hyperplasia, which was identified in 37.04% of all vessels, was observed in the majority of the graft samples removed from diabetic patients, who comprised 38.46% of the study population undergoing CABG. Intimal hyperplasia occurs as a response to physiological stimuli, as the tissue attempts to maintain normal conditions of flow and/or wall tension (Jones et al, 1972).

Excessive lipoprotein in the plasma may cause atherosclerosis, due to its tendency to accumulate in the hyperplastic intima (Allon et al, 2013). In the present study, the incidence of hyperlipidemia was 65.38% (17 cases), and the incidence of atherosclerosis in the ITA was 8.33% (2/24 cases), whereas no atherosclerosis was identified in the RAs. The prevalence of atherosclerosis in the two arteries may have been underestimated in this study because only the distal ends of arteries were examined, and atherosclerosis is a segmental disease.

When investigated using microscopy, 27.78% of the specimens were found to be normal, and 31.48% were mildly to moderately fibrotic. On histopathological evaluation, vascular medial fibrosis of the tunica media was found to be present in all types of vessel grafts, suggesting that fibrosis is a global process that occurs regardless of the involved vessel. Among all cases of medial fibrosis, the SVs accounted for 41.17% (7 cases), compared with 29.41% (5 cases each) by the RA and ITA, respectively.

Medial vascular fibrosis increases the risk of cardiovascular events by contributing to the stiffening of vessels and reduced vascular compliance (Sulikowski et al, 2010). In this study, the known cardiovascular risk factors, hypertension (65.38%), DM (38.46%) and age (61.54%) were present, and may be associated with increased vascular fibrosis. Some authors (Selvin et al, 2010) reported that vascular fibrosis is a global process associated with diseases of elevated pulse pressure and aging.

In our study, the two indices, ITI and the percentage of luminal narrowing, were used for morphometric analysis of vascular conduits. The calculated ITI was significantly higher in ITAs (1.66) than in RAs (0.95) and SVs (0.37). So, ITI assessement showed that ITAs had a thicker intima than that of RAs and SVs. These measurements correspond to different degrees of luminal narrowing in the vascular conduits, resulting that ITI was of third degree in ITAs and of second degree in RAs and VSs. These data indicate that SVs have the least severe pre-existing lesions before CABG surgery.

The percentage of luminal narrowing indicates the degree of intimal thickening, including atherosclerosis. The area of the intima, rather than the thickness, was measured to allow eccentric or irregular lesions to be evaluated more accurately. The percentage of luminal narrowing is considered to be the parameter most useful for comparing intimal thickening in vascular beds of different types (Thiene et al, 1980).

The authors of the present study hypothesized that both ITI and IMR may be used to compare the intimal layer thickness in various vascular diseases, which is consistent with another study (Ruengsakulrach et al, 1999). In our previous study we demonstrated that the ITI method was more accurate, since it uses areas, rather than dimensions, such as vascular layer thickness.

There were several limitations to the present study. First, only the changes of ITA, RA and SV histology immediately prior to CABG were evaluated, and secondly, the patients were not clinically followed up, and so it was not possible to correlate CABG outcome with the histopathological findings.

Final remarks

The ITI and measurements of intimal and medial areas may serve as reference points in the follow-up assessment of arterial and venous conduit patency. Factors identified in the patients that are likely to be significant predictors of lesion severity (associated with, for example, intimal hyperplasia and atherosclerosis) in the CABGs were hyperlipidemia, arterial hypertension, smoking, age and DM.

III.3. Inflammatory myocardial diseases

III.3.1. Introduction

Myocarditis is the inflammation of the heart muscle and an important cause of acute heart failure, sudden death, and dilated cardiomyopathy. Myocarditis can be classified in a number of different ways.

Etiologically, myocarditis can be a manifestation of almost every infectious agent. Viruses account for most cases of myocarditis or inflammatory cardiomyopathy, which could induce an immune response causing inflammation even when the pathogen has been cleared (Fairweather et al, 2005). Bacteria less frequently cause myocarditis than viruses. Invasion of the bloodstream by any bacterial pathogen can result in myocardial seeding and microabscesses. Parasites are a major cause of eosinophilic myocarditis worldwide being the principal cause of developing chronic heart failure by ischemia and arrhythmia. Although myocarditis is commonly used for viral myocarditis, there are numerous other heart conditions leading to myocardial inflammation, including acute ischemic injury, infiltrative diseases, allergies, and toxic or mechanical injuries.

Histologically, myocardial inflammation is accompanied by (Aretz et al, 1987; Feldman & McNamara, 2000) hyperemia and leukocytes infiltration, as well as edema and necrosis, as typical features of reversible or irreversible cellular injury. This inflammation is necessary to limit tissue damage, initiate the healing process, and eliminate dying cells and debris after injurious stimuli in the heart (Medzhitov et al, 2010).

On the other hand, persistent noxious stimuli, conditions including infection and adverse immune responses can partly explain the pathophysiology of inflammation and tissue injury in the myocardium. The inflammatory response promotes the recruitment of leukocytes and plasma proteins to the heart tissue (Medzhitov et al, 2008). These constituents, in turn, contribute to a transient decline in function of the tissue, alter homeostasis, and may hasten the progression of disease to its sequelae, dilated cardiomyopathy (DCM) and congestive heart failure (Cihakova & Rose, 2008).

However, clinically, myocarditis has a wide spectrum of clinical presentations and causes that make the consensus on diagnosis and universal classification scheme difficult. In an attempt to incorporate the clinical presentation and histopathologic findings into diagnosis, myocarditis is divided into four categories: acute myocarditis, fulminant myocarditis, giant cell myocarditis, and chronic active myocarditis. This classification incorporates the chronicity and

severity of disease, as well as potential specific treatment strategies. This is particularly important for giant cell myocarditis, which requires early endomyocardial biopsy and consideration for immunosuppressive therapy (Cooper et al, 2012).

In the last few years, advances in noninvasive techniques such as cardiac magnetic resonance have been very useful in supporting diagnosis of myocarditis, but toxic, infectious-inflammatory, infiltrative, or autoimmune processes occur at a cellular level and only endomyocardial biopsy can establish the nature of the etiological agent.

So, the disease process in myocarditis can only be diagnosed by histological investigation of very small pieces of heart tissue (biopsies). A biopsy is performed when myocarditis is suspected and when making the diagnosis of myocarditis may impact treatment options or prognosis (expected outcomes as in life-threatening outcomes). It is recommended that if a patient has an indication (reason) for an endomyocardial biopsy and they are at a medical center where this expertise is unavailable, the patient should be transferred to a medical center with this expertise (Cooper et al, 2007).

From the rare proposed diagnostic etiologic entities in myocarditis, such as cardiac echinocosis and sarcoidosis, only in sarcoid myocarditis EMB is recommended. In the case of sarcoidosis, a rare immune myocarditis, the endomyocardial biopsy provides a definitive etiological diagnosis that can lead to specific treatments such as antiviral or immunosuppressive therapy (Cooper, 2012). Cardiac hydatid cyst is an extremely rare disease. The diagnosis of cardiac hydatid disease is based on the combination of clinical suspicion, serologic tests and cardiac imaging. Histological study has only research significance.

Cardiac sarcoidosis

Sarcoidosis is a rare granulomatous disease of unclear etiology. It is a fatal disease when sarcoidosis involves the heart (Schulte et al, 2005). Cardiac sarcoidosis (CS) is an unusual form of granulomatous *myocarditis.* The clinical evidence of myocardial involvement is present in approximately 5 % of patients with sarcoidosis. Patients who present with apparently chronic dilated cardiomyopathy and new ventricular arrhythmias or second-degree or third degree heart block or who do not have a response to optimal care are more likely to have cardiac sarcoidosis (Dubrey et al, 2010). Sarcoid heart disease should be considered in the evaluation of an otherwise healthy young or middle aged person with cardiac symptoms or in a patient with known sarcoidosis who develops arrhythmias, conduction disease, or heart failure. Endomyocardial biopsy shows characteristic non-caseating granulomas. However, the diagnosis can also be supposed if there is a tissue diagnosis of sarcoidosis from an extracardiac source in the presence of a cardiomyopathy of unknown origin (Chapelon-Abric et al, 2004).

Cardiac echinococcosis

Cardiac echinococcosis is a rare manifestation of cyst echinococcosis with a reported prevalence of 0.5–2% (McManus et al, 2003). Cardiac and vascular involvement is infrequent in classical cystic echinococcosis (CE), but when it occurs, it tends to have an earlier development and is associated with complications that may be life threatening (Bashour et al, 1996). The clinical picture and complications vary according to cyst location. In early CE patients can be asymptomatic. In advanced CE, the main clinical manifestations include thoracic pain or dyspnea, mimicking cardiac ischemia. Usually, cardiac hydatid cyst is suspected on echocardiography and confirmed by magnetic resonance imaging. Cardiac CE usually requires complex surgery, so in low-income countries the outcome is frequently fatal

(Neuville et al, 2010.). Isolated cardiac CE may be cured after surgery, while endovascular extracardiac involvement is associated with severe chronic complications.

Objectives

Our diagnostic experience includes the evaluation of rare cases whose understanding has contributed to current knowledge acquired in the field, as useful additional data.

III.3.2. Cardiac sarcoidosis - a possible cause of death

Case presentation

We describe the case of a 72 year old male known for a few months with pulmonary and lymph node stage II sarcoidosis who arrived to our hospital with moderate effort dyspnea. Cardiac evaluation revealed multiple lesions (degenerative aortic valve disease, calcification of the posterior mitral annulus) which required surgical intervention for aortic biological prosthesis implant. We harvested a myocardial fragment and hilar and thymic lymph nodes.

Macroscopically, the lymph node biopsy revealed a modified appearance: enlarged sclerotic nodes, black in color on the cross section (fig. III.3.2.1).

The microscopic examination of the cardiac biopsy revealed various aspects: subendocardial epithelioid granulomas, inflammatory foci and subendocardial fibrosis (fig. III.3.2.2.a), and intramyocardial inflammatory infiltrates with architectural disturbance (fig. III.3.2.2.b).

Histologically, the thymus lymph nodes presented granulomas with giant cells and tendency to fibrosis (fig. III.3.2.2.c).The microscopic appearances of the hilar lymph nodes variated from lymph node sclerosis with few peripheral giant cell granulomas (fig. III.3.2.2.d) to lymph nodes with active inflammatory processes and many giant cell granulomas.

Although the myocardial biopsy of interventricular septum established the diagnosis of cardiac sarcoidosis, corticotherapy was delayed untill cardiac pathology recovery.

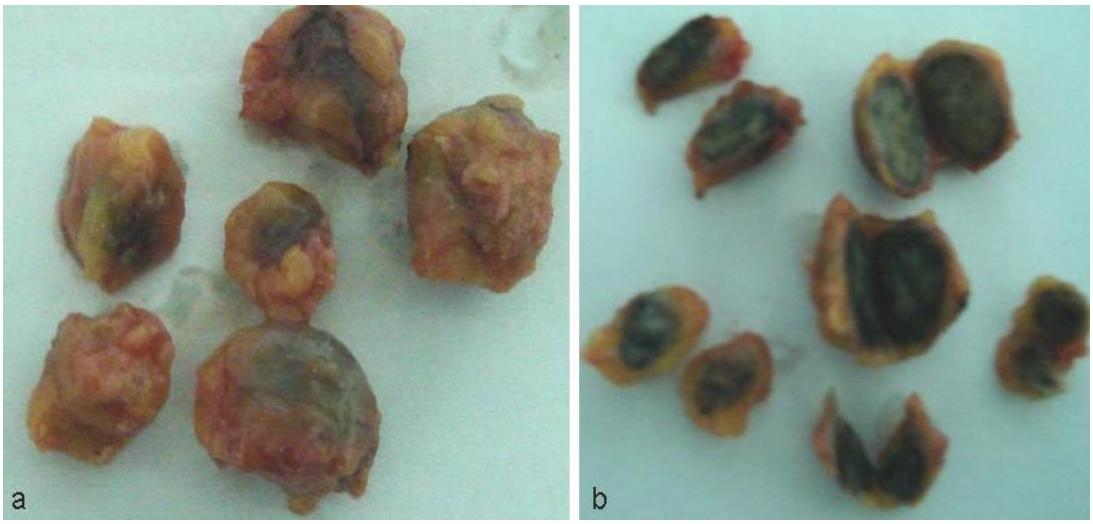

Figure III.3.2.1 Hilar and thymic lymph nodes showing enlarged sclerotic nodes (a), black in color on the cross sectional area (b), due to associated anthracosis.

Six months post surgey intervention, the patient developed progressive heart faillure with normal troponin T value, left ventricular regional motility abnormalities while

corticotheraphy has been stoped. Persistent atrial fibrillation has been chemically and electrically reduced.

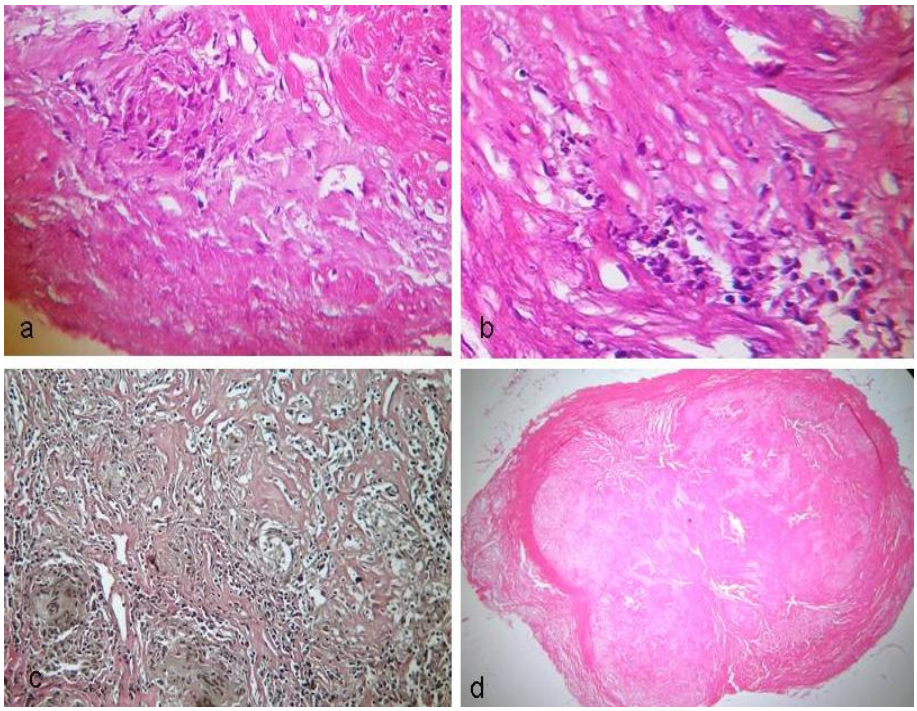

Figure III.3.2.2. (a) Subendocardial epithelioid granuloma with tendency to fibrosis, HE x 40; (b) Focal intramyocardial inflammatory infiltrate, HE x 40; (c) Active thymic lymph node with giant cell granulomas, VG x20; (d) Sclerotic hilar lymph node, HE x10.

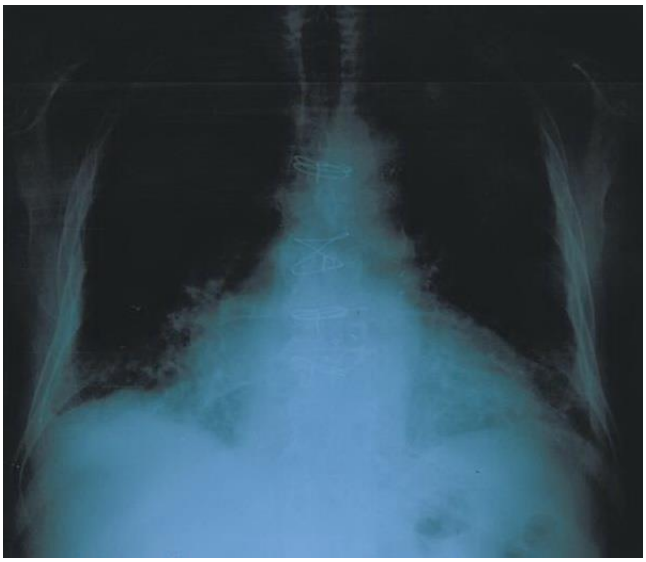

Figure III.3.2.3. Chest X ray indicates global cardiomegaly, bilateral increased hilar and basal pulmonary markings.

One year post intervention, the cardiac examination revealed multiple abnormalities: global cardiomegaly, bilateral stiffen hilar and basal pulmonary markings on chest x-ray (fig. III.3.2.3.), global cardiac enlargement predominant on the left side, moderate systolic dysfunction of left ventricle and EF under 35% on the echocardiography.

The regional motility abnormalities interested the ½ of apical interventricular septum, left ventricular apex and ½ apical of antero-lateral wall which were diskinetic and the inferior wall which was akinetic. EKG displayed SR with 60-100 bpm, no arrhythmia and a complete LBB; coronary angiography revealed slightly irregularities of the coronary wall and the blood examination added an active inflammatory syndrome (ESR-mmHg, fibrinogen-584 mg%, high C-reactive protein) with secondary normochrome anemia.

Discussion

Cardiac sarcoidosis (CS) was first described clinically, about 100 years ago (Bernstein et al, 1929; Fleming, 1986) and pathologically, 40 years later (Gozo et al, 1971). It was appreciated on autopsy studies that about 5% of systemic sarcoidosis involves the heart (Silverman et al, 1978; Hagemann et al, 1980; Iwai et al, 1994). Further, myocardial biopsy studies proved higher incidence (Ayyala et al, 2005; Kim et al, 2009).

Myocardial sarcoidosis diagnosis is difficult and frustrating. Clinical manifestation of CS depends on the localization and extension of granulomatous inflammation and signs and symptoms that vary from arrhythmia, cardiac block to untreatable heart failure. Even more, cardiac involvement appears after or simultaneous with lungs or other organ involvement.

The diagnosis is relatively easy if the cardiac manifestation appears to a patient with multisystemic sarcoidosis. When cardiac dysfunction is the only sarcoid cardiac manifestation, the diagnosis is often unconfirmed because there is a lack of specific diagnosis tests. CS needs extensive investigations of high sensitivity (Suzuki et al, 1994; Burstow et al, 1989; Kiuchi et al, 2007 Hulten, 2016). MRI is included in CS diagnosis and monitoring (Hulten, 2016).

In our case, we performed cardiac presurgical evaluations which included ECG, 24h Holter monitoring, echocardiography and coronary arteriography. The correct location of granulomas leads to a high quality myocardial biopsy. Although the myocardial endobiopsy is preferable for the CS diagnosis, it proves low sensitivity (20%) if it is not well guided (Matsuki et al, 2000). The search for an easier CS diagnosis test is still on.

A Japanese expert group (Uemura et al, 1999) proposed a diagnosis guide for CS which includes clinical and histological criteria: A-major; B-minor criteria.

A. Histological diagnosis of CS on myocardial endobiopsy or surgical biopsy consists of distinguishing of non caseous epithelioid granulomas.

B. Clinical diagnosis: (a) - atrioventricular block, ventricular tachycardia, premature ventricular contraction (>Lown 2), abnormal Q waves and ST spaces on ECG; (b) - abnormal wall contraction, regional wall rarefaction or LV dilatation; (c) - thalium 201 perfusion deffect on myocardial scintigraphy; (d) - abnormal intracardiac pressure, abnormal LV wall contraction or low LV EF; (e) - uncharacteristic histological features: interstitial fibrosis or moderate degree cellular infiltrates.

A patient with known diagnosis of extracardiac sarcoidosis is suspect of CS if there are present A and one or two of B-E criteria. Applying this protocol in our case we had a doubtless diagnosis of cardiac involvement in the existing systemic disease context. Nowadays, the group of Japanese experts improved the diagnosis of CS by updating the diagnostic guideline for current use in clinical practice (Kusano and Satomi, 2016).

The prognosis of the patients with CS was not well estimated. Some authors appreciated 2 years survival from the beginning of cardiac signs (Roberts et al, 1997). Other authors

reported that patients survived 5 years from the first clinical disease presentation (Flemming et al, 1994).

Many of these conclusions have been made before the development of implantable defibrillators, aggressive use of corticosteroids immunosuppressive and immunomodulatory agents. So, two demands are required for increasing life expectancy: the diagnosis must be correct and precocious and the treatment aggressive and efficient.

In Japan, the majority of death associate to sarcoidosis are caused by CS that is why the cardiac involvement diagnosis is necessarily (Sharma et al, 2003; Shimada et al, 2001). Usually, death is caused by congestive heart failure and less by sudden death. A more common anomaly is high degree atrioventricular block.

Final remarks

CS remains a challenge despite the progress in precocious myocardial disease diagnosis. Choosing the right therapeutical algorithm for sarcoidosis requires a certain invasive and noninvasive sustained diagnosis.

III.3.3. Cardiac echinococcosis – a cause of unexpected death

Case presentation

A 50 year-old man, from a rural zone, sheep-raising area, was admitted in hospital for evaluation of an atypical anterior chest pain by only 4 weeks. The lab tests showed a moderate inflammatory syndrome and lack of eosinophilia. Ischemic changes in T wave were found on ECG recording. The echocardiography has evidentiated a moderate cardiomegaly.

CT scan showed the presence of a septal cystic mass. The patient died at 6 days from hospital admission due to a progressive cardiovascular collapse.

On gross examination of the heart, we detected a cystic lesion of 6 cm in diameter located at the level of antero-inferior part of the interventricular septum. The cyst was typically composed of a stratified wall: fibrous adventicea, an anhiste and proligere membranes.

Macroscopically, we identified also two openings: (a) one more evident, oval in shape, of about 0.5 cm in diameter, connecting the cyst to the left ventricular cavity; we found the presence of small daughter cysts into ascending aorta, as well, confirming the systemic embolism; (b) the other opening was an irregular right ventricular endocardial fissure, of about 0.3 cm length, permitting the hydatid fluid penetration in the right ventricle and in this way in the pulmonary circulation; this aspect was confirmed histologically, by the presence of anhiste membrane emboli into peripheral pulmonary vessels.

On microscopic examination of the heart, the internal germinative layer with daughter cysts (fig. III.3.3.1), the external laminated layer, and the cystic fibrotic wall were detected. We revealed also an adjacent myocardial area presenting a moderate inflammatory cellular infiltration, mainly composed of eosinophils, lymphocytes and plasma cells (fig. III.3.3.2), in parallel with myocyte atrophy and diffuse interstitial fibrosis, as signs of chronic myocardial ischemia (fig. III.3.3.3). On microscopic analysis of the lung sections, the presence of multiple emboli into small peripheral pulmonary vessels were detected, represented by fragments of anhiste membranes filling the peripheral pulmonary arterioles (fig. III.3.3.4).

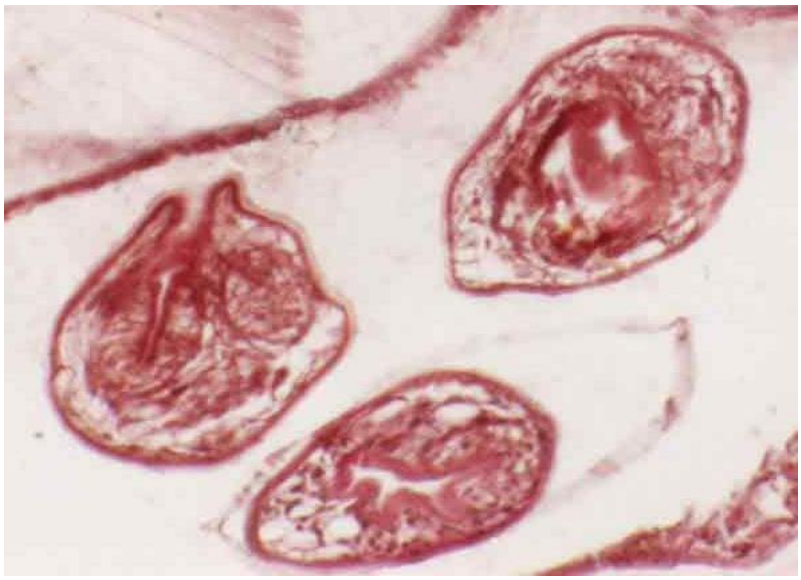

Figure III.3.3.1. The internal germinative layer with daughter cysts (HE X40)

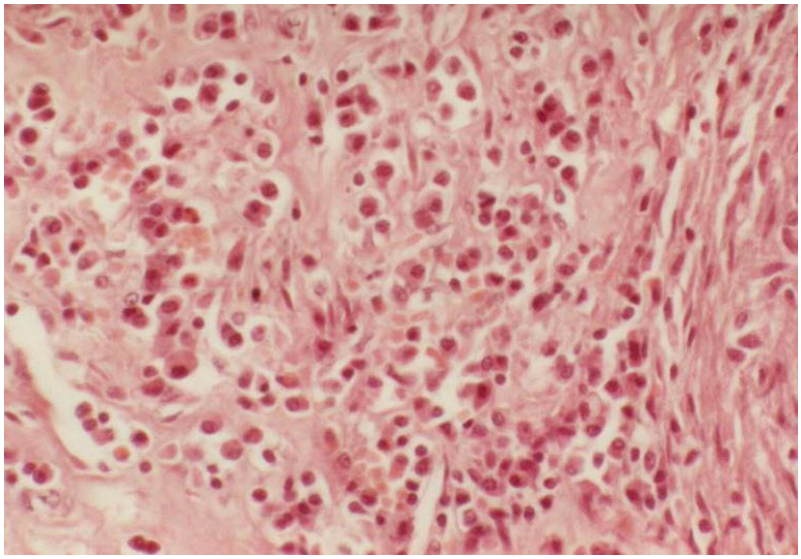

Figure III.3.3.2. Pericystic chronic inflammatory infiltrate (HE X20)

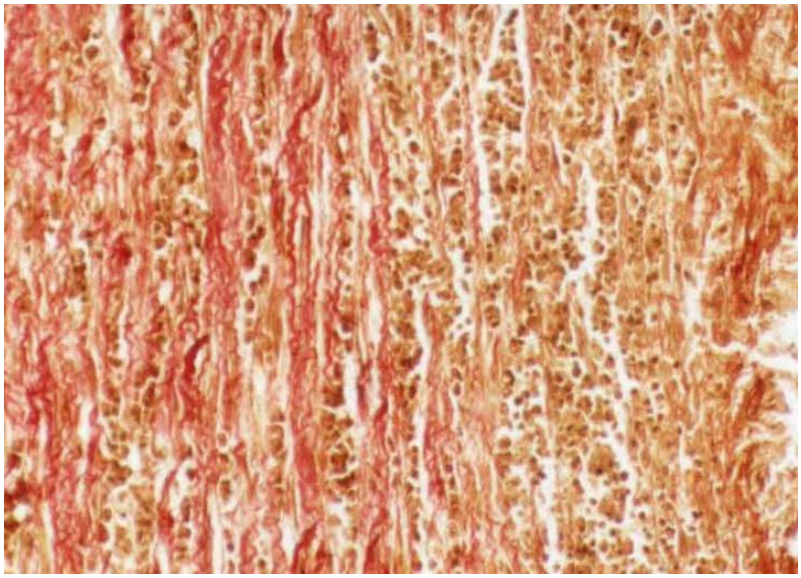

Figure III.3.3.3. Pericystic chronic inflammatory infiltrate and fibrosis (VG X20)

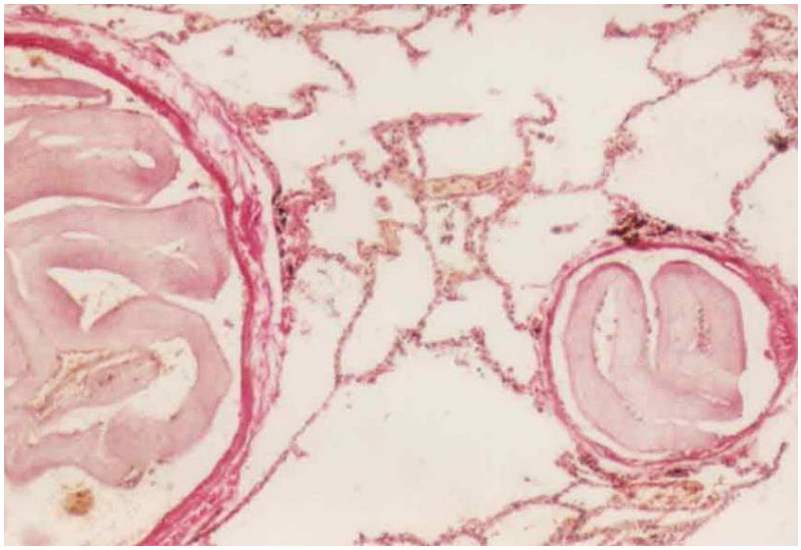

Figure III.3.3.4. Pulmonary emboli with anhiste membranes (VG X10)

No other gross or microscopical pathological lesions were detected in the chest or abdominal organs in the systematic examination.

The cause of death in this case was referred as an anaphylactic shock due to intravascular spread of the hydatid cyst content.

Discussion

Hydatid disease is a parasitic infestation caused by a tapeworm. Echinococcus is endemic in many parts of the world, but cardiac involvement with hydatid cyst is uncommon, occurring in less than 2% of cases (Jannati et al, 2006).

Cardiac involvement by hydatid cyst is rare. The myocardium of the interventricular septum or left ventricular free wall is the usual site for cardiac involvement of hydated cyst,

but occasionally right atrium or ventricle may be involved, as well (Murphy et al, 2005). Cardiac cysts are usually associated with fatal complications (Kammoun et al, 2000).

Cardiac echinococcosis is mostly symptomatic. These symptoms include angina, dyspnea and palpitation (Depaulis et al, 1999). The ischemic changes are due to compression or complete obstruction of the coronary arteries present in the area of the cyst (Telli et al, 2001). Therefore, a complete cardiac evaluation should routinely be performed in all patients with cardiac echinococcosis, including coronarography. A myocardial cyst may degenerate and calcify or rupture into pericardium or heart chambers. Rupture of the cyst is the most serious complication, inducing either acute or advanced chronic constrictive pericarditis, either systemic or pulmonary emboli (Tejada et al, 2001; Kaplan et al, 2001). The passing of the hydatid fluid into the circulation may also produce fatal circulatory collapse in response to anaphylactic reactions to protein constituents of the fluid (Guven et al, 2004). Few patients infected with cardiac hydatid cyst may not have any obvious clinical symptoms (Guven et al, 2004; Kucukarslan et al, 2005; Markatis et al, 2020).

Mortality rates of asymptomatic cases of cardiac involvement following perforation of the cyst are relatively high, because of acute anaphylactic reaction and cardiogenic shock, which may occur in these patients (Jannati et al, 2006).

Usually, cardiac hydatid cyst is suspected on echocardiography and confirmed by magnetic resonance imaging.

Cardiovascular CE usually requires complex surgery (Neuville et al, 2010). Isolated cardiac CE may be cured after surgery, while endovascular extracardiac involvement is associated with severe chronic complications. The new experiences suggest that benzimidazole derivatives are useful drugs for the treatment of hydatid cyst (Oliviero et al, 2000; Tuncer et al, 2010).

Despite the availability of valuable medical treatments, the surgical excision is generally recommended for both symptomatic and asymptomatic patients. This is because of the high risk of cyst rupture and its serious consequences. So, the surgical cyst removal can prevent the systemic emboli and fatal circulatory collapse, which may occur in such cases (Neuville et al, 2010).

Hydatid disease is a dangerous condition because of risk for complications. We commented the death as the result of anaphylactic phenomena following the rupture of echinococcal cyst, by intravascular spread of cyst content.

Final remarks

Primary echinococcus infection of the heart is a rare type of cystic echinococcosis. CE should be included in the differential diagnosis of cardiovascular disease in patients from endemic areas. CE is a neglected disease and further studies are necessary in order to make more definite management recommendations for this rare and severe form of the disease.

III.4. Primary cardiomyopathies

III.4.1. Introduction

For more than 30 years, the term *cardiomyopathies* has been used to describe disorders of the heart with particular morphological and physiological characteristics. So, heart muscle diseases have been classified into primary or idiopathic myocardial diseases

(cardiomyopathies) and secondary cardiac disorders that have similar morphological appearances (Richardson et al, 1996).

Recently, an expert committee of the American Heart Association proposed a new scheme in which the term primary is used to describe diseases in which the heart is the sole or predominantly involved organ and secondary to describe diseases in which myocardial dysfunction is part of a systemic disorder. Consequently, cardiomyopathies were classified as primary (i.e., genetic, mixed, or acquired) or secondary (e.g., infiltrative, toxic, inflammatory) (Maron et al, 2006).

The four major types are dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC) (Kaski & Elliott, 2007).

DCM, the most common form, affects five in 100,000 adults and is the third leading cause of heart failure. Dilated cardiomyopathy (DCM) in adults is most commonly caused by CAD (coronary artery disease or ischemic cardiomyopathy) and hypertension, although viral myocarditis, valvular disease, and genetic predisposition may also play a role (Maron et al, 2006).

HCM is the leading cause of sudden death in athletes with an incidence of one in 500 persons (Maron et al, 2003). HCM is caused by 11 mutant genes with more than 500 individual transmutations. The most common variation involves the beta-myosin heavy chain and myosin-binding protein C (Maron et al, 2003).

RCM and ARVC are rare, and their diagnoses require a high index of suspicion. (Kushwaha et al, 1997; Buja et al, 2008).

RCM is an uncommon form that occurs when the ventricles become too stiff to contract. This is often the result of an infiltrative process, such as sarcoidosis, hemochromatosis, amyloidosis, and abnormalities related to desmin (a protein marker found in sarcomeres) (Kushwaha et al, 1997).

ARVC is an autosomal dominant, inherited disorder of the muscle of the right ventricle. In arrhythmogenic right ventricular cardiomyopathy, the myocardium is replaced by fibro-fatty tissue. The same infiltrative process may also affect the left ventricle (Buja et al, 2008).

Diagnostic evaluation

The most common clinical presentation in patients with cardiomyopathy is heart failure. The evaluation for underlying causes of heart failure includes a thorough history and physical examination with baseline chemistries, including B-type natriuretic peptide (BNP) levels, echocardiography and electrocardiography (ECG); chest radiography should be performed on patient's initial presentation.

Cardiomyopathies (CMPs) are grouped into specific morphological and functional phenotypes; each phenotype is then sub-classified into familial and non-familial forms (Fig. III.4.1) (Burkett & Hershberger, 2005; Maron et al, 2003; Thiene et al, 2004; Elliott & McKenna, 2004; Kushwaha et al, 1997; McKenna et al, 1994).

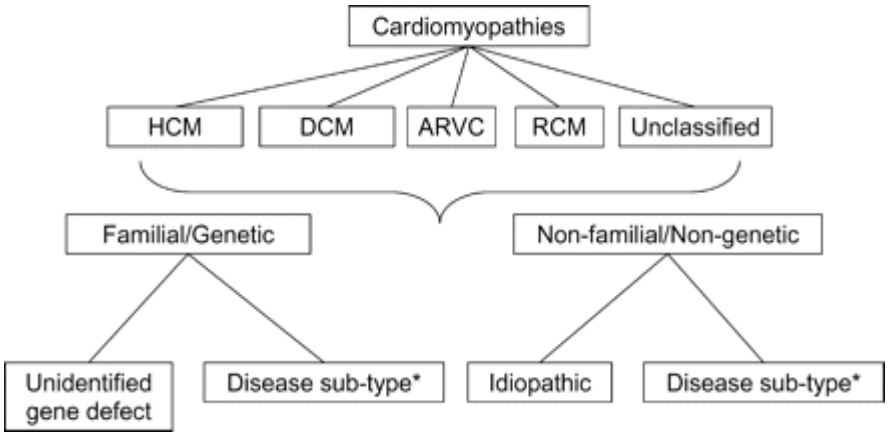

Figure III.4.1- Summary of proposed classification system. ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; RCM, restrictive cardiomyopathy

In this context, the familial form refers to the occurrence, in more than one family member, of a phenotype that is caused by the same genetic mutation. Non-familial cardiomyopathies are clinically defined by the presence of cardiomyopathy in the absence of such diseases in other family members (based on pedigree analysis and clinical evaluation). They are subdivided into idiopathic (no identifiable cause) and acquired cardiomyopathies in which ventricular dysfunction is a complication of the disorder rather than an intrinsic feature of the disease.

From the entire cardiomyopathy family, ARVC is a rare cardiac disorder characterized by gradual replacement of myocytes of the right ventricular wall by adipose and fibrous tissue (Anderson et al, 2006). Described in 1977 (Marcus et al, 1995), ARVC is considered a potential cause of lethal cardiac disease. This disorder usually involves the right ventricle and has been associated with arrthymia, heart failure, and sudden death (Thiene et al, 1988).

Objectives

The aim of this study is to present the case of a professional athlete whose death was sudden and caused by an undiagnosed ARVC. Our unique experience, when combined with a literature review, permits a composite clinical profile of this condition in the adult.

III.4.2. Arrhythmogenic Right Ventricular Disease

Case presentation

A 21-year-old male, with no history of cardiovascular diseases or previous exercise related cardiac symptoms, was unresponsive to cardiopulmonary resuscitation maneuvers on March 2011. The patient died during sport activity and nobody was able to inform on the sincopal episode duration. There was no history of heart disease or cardiac sudden death among members of the family, either.

Grossly, at necropsy, all the cardiac chambers were enlarged and the heart weight was increased, having around 400g. The coronary arteries were normal. The left ventricular wall was 7 mm thick whereas the right ventricular wall was very thin (1 mm) and infiltrated by adipose tissue.

The histology of the free wall of the right ventricle clearly showed transmural fibrofatty replacement (fig. III.4.2.1).The pathological process, extending from the subepicardium to the endocardium in a wave-front pattern, presented a lace-like appearance.

On microscopic examination, the remaining cells were either hypertrophied or attenuated (fig. III.4.2.2.a) and presented many other lesions, consisting in wavy cell elongation (fig. III.4.2.2.b), contraction band necrosis (fig. III.4.2.2.c) and focal segmentation of the hypercontracted myofibers (fig. III.4.2.2.c), as a substrate of ventricular fibrillation. The tissue samples from the left ventricle wall showed a heterogeneous picture with variable degrees of myocardial injury and repair including acute necrosis with inflammatory infiltrates (fig. III.4.2.3.a), subacute damage with active fibrosis and adipocytes replacing myocytes (fig.III.4.2.3.b), along with chronic damage with mature fibrous tissue and adipocytes surrounding surviving residual myocytes (fig. III.4.2.3.c).

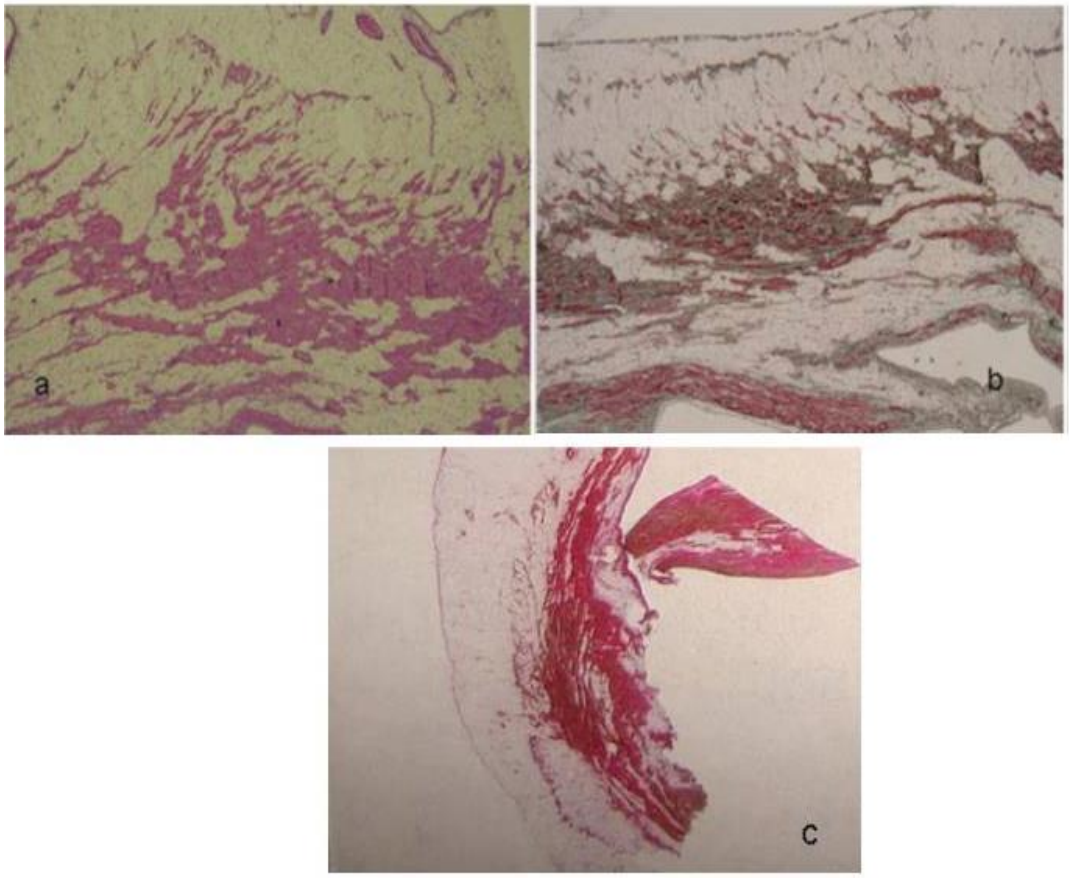

Figure III.4.2.1. Right ventricular wall with transmural fibro-fatty replacement (a-Hematoxylin-eosin- HE; b-Masson; c-Van Gieson staining)

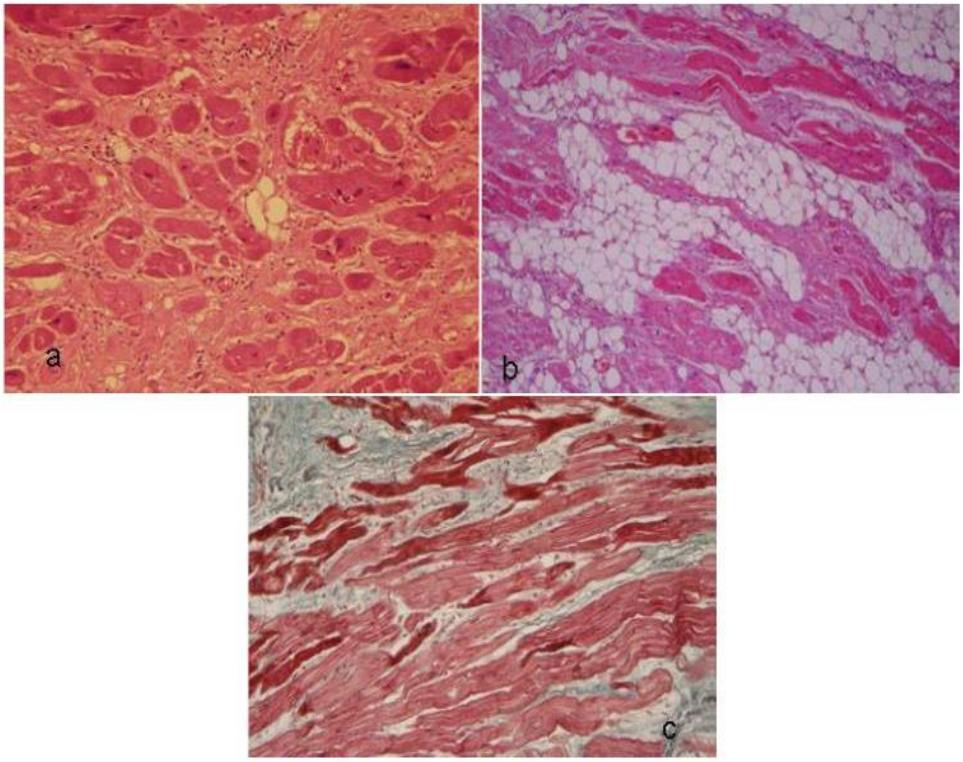

Figure III.4.2.2. a-Hypertrophied and atrophied cardiomyocytes and inflammatory infiltrate (HE); b-Wavy elongated cardiomyocytes (HE); c-Contraction band necrosis (Masson staining)

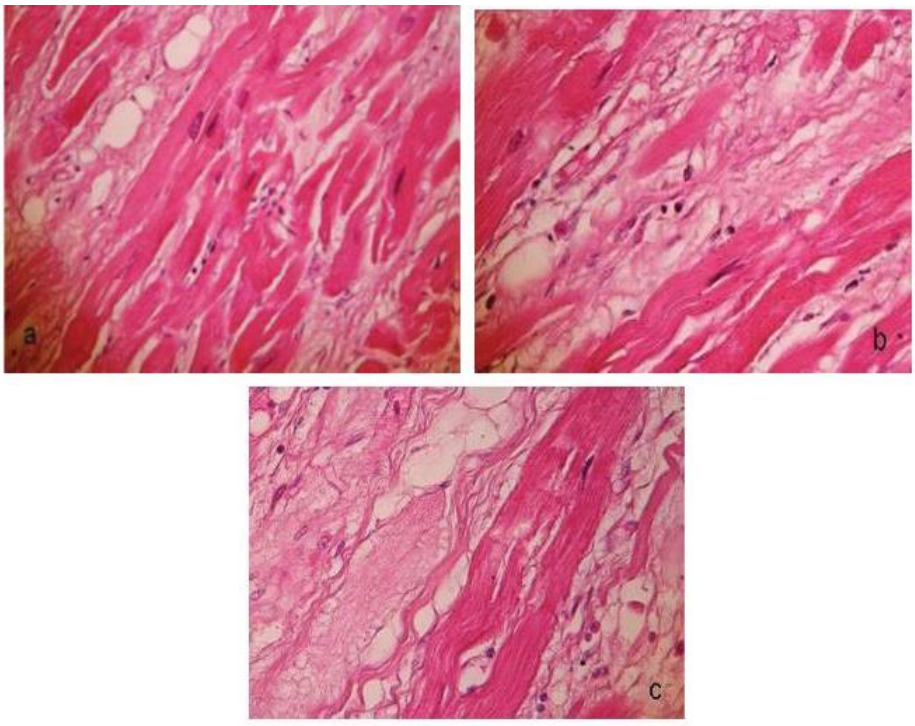

Figure III.4.2.3. Cardiomyocyte segmentation and contraction band necrosis Hematoxylin-eosin staining-a; b; c;

Discussion

ARVC is a primary myocardial disorder, characterized by a high incidence of arrhythmias and sudden cardiac death. It is listed among cardiomyopathies in the WHO classification (Richardson et al, 1996) and is defined by transmural fatty or fibro-fatty infiltration of the right ventricle. Arrhytmogenic cardiomyopathy is a cause of sudden death. In cardiomyopathies, the highest frequency (50%) of sudden death was observed in hypertrophic cardiomyopathy, followed in decreasing order by dilated cardiomyopathy (14%), while sudden death was rarely reported in restrictive cardiomyopathy. In arrhytmogenic cardiomyopathy, a high frequency of sudden death was noted without of percent figure offered (Barolldi et al, 2001).

As in this case, some authors noted (Thiene et al, 1988) that ARVC sudden death is more likely in young adults then in children, and males are more susceptible to that event than females. It is well known, that arrhytmogenic cardiomyopathy mainly affects the right ventricle, with partial and total atrophy of the myocardium and fatty or fibrofatty replacement. It is also noted that a phase of active lymphocytic myocarditis apparently precedes myocyte necrosis or apoptosis (Thiene et al, 1988). About 50% of cases have left ventricle involvement, including adolescents (Anderson, 2006). In our case, we found the involvement of both two ventricles. We consider that the disease progression can lead to diffuse right and left ventricle free wall involvement, but even in advanced disease, the interventricular septum tends to be spared, because of lacking of subepicardial area, as a beginning ARVC focus.

Sudden death in this disease is related to dysrhythmias generated at the junctional regions between atrophic and normal myocardium, hence the name of arrhytmogenic. The disease name reflects the usual predominant involvement of the right ventricle, but increasing recognition of biventricular involvement explains adoption of the broader term of arrhythmogenic cardiomyopathy (Fontaine et al, 1995; Sen-Chowdhry et al, 2007). In this case, the right ventricle was dilated and its free wall was yellowish and partly translucent. Histologically, the substitution of the right ventricle myocardium with fibro-adipose tissue associated with lymphocytic infiltrates was the ARVC hallmark. As other authors reported (Thiene et al, 1999), we showed an extensive transmural fibro-fatty replacement of the RV free wall, except for hypertrophied subendocardial myocytes.

But, fatty infiltration of the RV is not considered “per se” a sufficient morphologic hallmark of ARVC, because a certain amount of intramyocardial fat is normally present in the right ventricle antero-lateral and apical region even in the normal heart and increases with age and body size. Therefore authors consider that the presence of replacement-type fibrosis and myocyte degenerative changes are essential in providing a clear-cut diagnosis, besides remarkable fat replacement (Basso et al, 2005). The severely atrophied right ventricle myocardium replaced by fibro-fatty tissue should be regarded as a healing phenomenon following myocyte deaths (Basso et al, 1996).

Indeed, the fibrous tissue present in variable amounts is an essential part of the healing process and plays a fundamental role in the intraventricular conduction delay of the electrical impulse, which is at the basis of onset of life threatening arrhythmias. Death of single or multiple myocytes may be associated with inflammatory infiltrates (Thiene et al, 1991). It was suggested that right ventricular dysplasia may be a consequence of a previous myocarditis

(Basso et al, 1996). In our case, we have also described mononuclear inflammatory infiltrates in affected areas, but it is unclear whether this is a primary manifestation of the disease or develops as a secondary response to myocyte injury. As in other scenarios, we think that inflammation could play a pathogenic role in tissue injury and arrhythmogenesis, although this potential mechanism remains largely unexplored (Thiene et al, 2001).

Physiologically, the fibro-fatty replacement of the myocardium interferes with intraventricular conduction of the electrical impulse accounting for electrical impulse delay and onset of re-entrant phenomena which are the mechanisms of ventricular arrhythmias. That is why a more extensive quantitative study of myocardial functional lesions in this cardiomyopathy could help understanding the cause of cardiac arrest at autopsy (Corrado et al, 2001).

A very important issue is the postmortem recognition of cardiac arrest. It may have relevance from a forensic point of view, in establishing the cause of death. It is known that the heart may stop after ventricular fibrillation, generally preceded by a malignant arrhythmia, or in asystole as an end result of bradycardia, or in electromechanical dissociation, which is the loss of mechanical function despite a normal electrocardiogram (Barolldi et al, 2005).

At present, the morphologic background for different types of cardiac arrest is poorly defined, and apart from a few striking conditions (e.g., heart rupture plus tamponade), we cannot structurally diagnose the cause of a myocardial arrest (Maron & Spirito, 1998). As in this case, the main type of cardiac arrest predominant in sudden death is ventricular fibrillation resulting from arrhytmogenic conditions. The development of ventricular fibrillation is the result of imbalance between factors that enhance electrical synchrony and factors that decrease electrical asynchrony (Fineschi et al, 2006).

The questions are whether morphology of the electrocardiographic pattern exists, what causes it, and how it evolves. Nowadays, the segmentation of hypercontracted myofibers, considered artifacts in the past, is recognized as a possible agonal event related to ventricular fibrillation (Maron & Spirito, 1998). It was suggested, that various lesions may show correlation between myobreakup and electrocardiographic chaos: (a) bundles of hypercontracted myocells alternating with bundles of hyperdistended myocardial cells; (b) single or groups of hypercontracted myocardial cells disposed in line with hyperdistended ones; (c) intercalated discs between hypercontracted elements being widened, streched or segmented (Barolldi et al, 2005). In this case, we found segmentation of hypercontracted myofibers as a focal lesion, in left and right ventricles of the myocardium.

Another question is related to the stimulus for arrhytmogenic ventricular fibrillation. It is considered that impairment in myocardial metabolism could precede ventricular fibrillation. In many experiments, ventricular fibrillation was reduced or abolished by betablocking agents, pointing out an adrenergic stimulation role (Corrado et al, 2001). It was suggested that a single focus of myofibrillar breakup may correspond to an instantaneous ventricular fibrillation, while an extensive lesion may be associated with a relatively long-lasting malignant arrhythmia (Barolldi et al, 2005). We consider that segmentation and related findings seem to be reliable histological patterns for diagnosing cardiac arrest due to ventricular fibrillation.

Final remarks

Arrhythmogenic cardiomyopathy is a rare but important cause of sudden cardiac death in the young. Diagnosis of ARVC is a challenging problem and requires a comprehensive evaluation by both noninvasive and invasive testing.

III.5. Unexpected acute ischemia and Wolff–Parkinson–White syndrome

III.5.1. Introduction

Wolff–Parkinson–White (WPW) syndrome and myocardial infarction (MI) may be simultaneously present. In WPW syndrome, an aberrant working myocardium fascicle (improperly called "Kent fascicle") directly connects the atria to the ventricles apart from the specialized atrioventricular (AV) junction (Milliez & Slama, 2004). Such myocardial bridges between atrial and ventricular myocardium, accessory to the normal AV conducting tissue, have been reported either in otherwise structurally normal hearts or in hearts with congenital diseases, like Ebstein's anomaly. This aberrant fascicle of working myocardium can be located all around the left AV ring, where it is related to the attachment of the mural mitral leaflet and right AV ring (Torres, 2007). Accessory pathways in the septal area are less common and are located primarily on the right side. The "Kent fascicle" usually consists of a thin (mean 300 μm in thickness) bundle of working myocardium and does not possess decremental conduction properties (Kent, 1914).

It may serve not only as a bypass tract for ventricular preexcitation (thus explaining the short PQ interval and the delta wave of the QRS complex, the latter corresponding to early ventricular excitation), but also as a way for AV reentry circuit (usually retrograde), which causes supraventricular tachycardia, typical of WPW syndrome (Ozaydín, 2007).

The mismatch between the tiny anomalous fibers and the ventricular muscle bulk, in addition to fibrosis of the accessory fascicle, may explain impaired antegrade conduction and intermittent preexcitation.

Preexcitation syndromes are a not so minor cause of sudden death. The mechanism is believed to be paroxysmal atrial fibrillation, with nearly one to one conduction, because the aberrant fascicle lacks decremental properties, which may degenerate into ventricular fibrillation and cardiac arrest (Pietersen et al, 1992; Beatson et al, 2013). In these conditions, atrial alteration may trigger the onset of life-threatening lone atrial fibrillation.

The accessory fascicle along the AV sulcus is always located in the AV sulcus, much closer to the endocardium than to the epicardium; size and site are such that "Kent's fascicle" is easily controllable by endocardial transcatheter ablation, which is the current procedure to interrupt the preexcitation and to reestablish the sole regular electrical connection through the His bundle (Gallagher et al, 1976).

Wolff-Parkinson-White ventricular preexcitation is an uncommon congenital heart disease that affects 0.5-1‰ of live births. The risk of sudden death in patients is low and mainly related to the occurrence of atrial fibrillation. This may convert to ventricular fibrillation because of the short refractoriness of the AV accessory pathway, which allows transmission of more than 300 impulses per minute to the ventricles (Priori et al, 2001). Mahaim fibers, which connect the AV junction to the upper ventricular septum, may also participate in ventricular preexcitation (Demosthenes et al, 2017).

In WPW syndrome, the most common arrhythmias are benign, but sudden death may occur. The incidence of sudden death in patients with Wolff–Parkinson–White syndrome is estimated to be less than 1 per 100 patient-years of follow-up (Priori et al, 2001). In symptomatic patients, curative ablative therapy prevents recurrent arrhythmias, including atrial fibrillation (Fengler et al, 2007; Astorri & Pattoneri, 2006). In cases of sudden death in patients with known Wolff–Parkinson–White syndrome, histologic confirmation of the bypass tract is difficult, and the most important task facing the forensic pathologist is the exclusion of other potential causes of death (Gallagher et al, 1976).

Diagnosis

ECG changes during exercise stress testing, such as false-positive ST-segment depression and disappearance of the delta wave, are reported in patients with the Wolff-Parkinson-White pattern.

Although ST-segment depression, typical for ischemia, occurs in half of the patients with WPW syndrome, exercise testing is still an important tool in their evaluation. Data, other than ECG response, can be interpreted in the context of clinical history and physical examination findings to stratify the risk of coronary disease. Complete and sudden disappearance of the delta wave has been seen during exercise in 20% of patients with WPW syndrome and can identify those who are at low risk for sudden arrhythmic death.

Objectives

Although such pathways are rare, their unique properties make their diagnosis and treatment difficult. In this article we review the published evidence, and discuss the pathological characteristics of the WPW syndrome and MI association. This study undoubtedly yields many useful data for medical practice.

III.5.2. A peculiar association in a myocardial infarction

Case presentation

We report a recent myocardial infarction associated with WPW syndrome occurring in an athlete during sport training. The team colleagues described the patient having palpitations and exertional substernal chest pain lasting minutes and followed by syncope. ECG made by rescue team revealed deep Q waves in leads II, III, and aVF and delta waves in leads V2 and V3. The EKG findings were consistent with WPW syndrome rather than with myocardial infarction. He died shortly after hospital admission with no response to resuscitation efforts undertaken. Autopsy revealed a heart in normal shape, with no cavity dilatations. The pericardium, cardiac valves, endocardium, and coronary arteries were normal. Histological exam revealed contraction of apparently normal coronary artery and fractures of the contracted cardiomyocytes (fig.III.5.1.a, b). Foci of cardiomyocyte (CM) fracture in apparently normal CMs were also found. The dominant morphologic lesion was represented by CM death of various types, represented by infarct necrosis and contraction band necrosis (CBN).

Infarct necrosis, which is apparently the result of sudden nutrient flow reduction, was present in the posteroinferior wall, having a transmural extension. (fig. III.5.1 c). At periphery of infarcted area foci of undulated CMs in a fibrotic interstitium (fig.III.5.1d) were seen. Coagulative myocytolysis or CBN (fig. III.5.2 a), usually caused by adrenergic stimulation, had a focal distribution being associated in some areas with inflammatory microfoci (fig.III.5.2

b) and in other areas with large zones of hemorrhagic necrosis (fig. III.5.2 c), representing areas of reperfusion injuries.

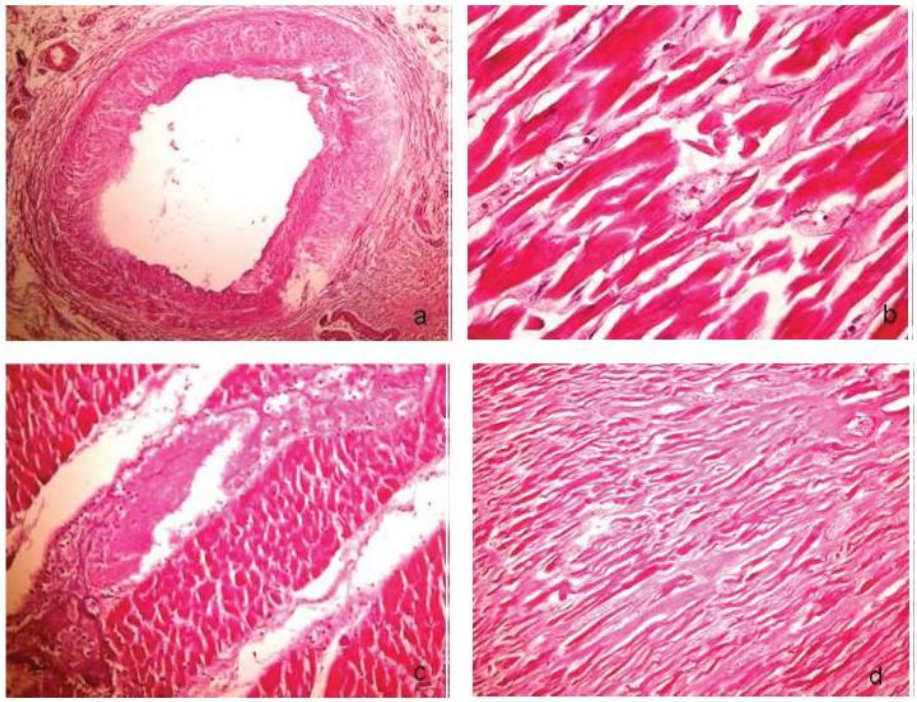

Figure III.5.2.1. a-contracted coronary artery (HE, x20); b-fractures of the contracted cardiomyocytes (HE, x40); c-area of infarct necrosis (HE, x 20); d-undulated cardyomyocytes and intercellular fibrosis (HE, x10).

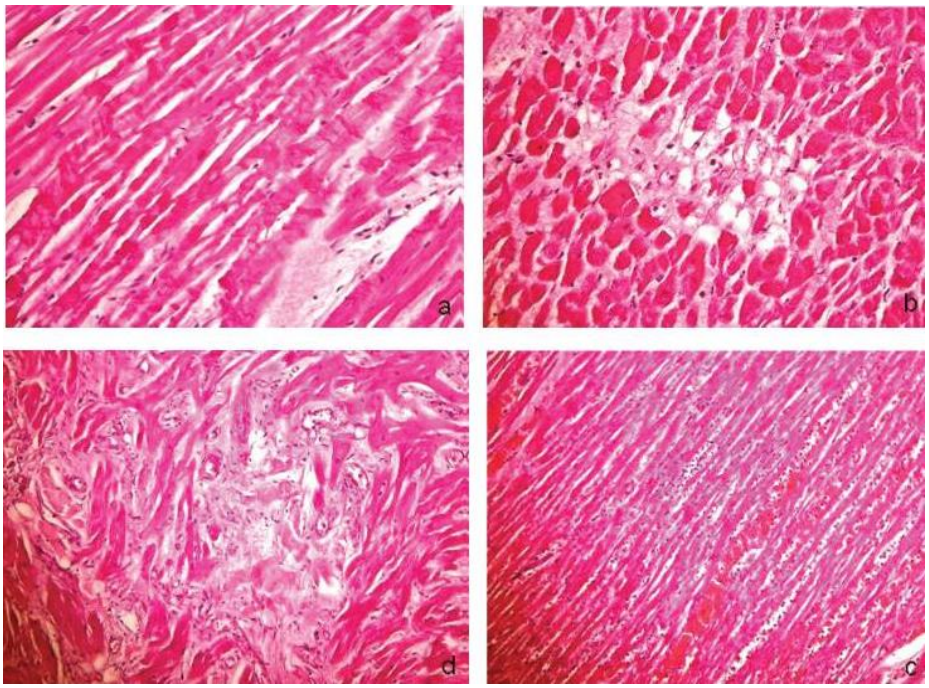

Figure III.5.2.2. a-Cardiomyocytes with CBN (HE, x20); b - Coagulative myocytolysis and macrophage digestion of the necrotic cells (HE, x20); c-Ischemic reperfusion injury (HE, x10); d-Myocardial dissaray (HE, x10).

No sign of colliquative myocytolysis, which is generally linked to catecholamine depletion, and no signs of apoptotic-like lesions were found. Focally, the myocardial interstitium was enlarged by replacement of damaged CMs with fibrous tissue of different histological ages, having either a predominant matriceal component with few wavy fibers, or a prominent collagen component corresponding to chronic cardiac ischemia. In few areas, this progressive connective tissue repair with neo-angiogenesis component resulted in CM architectural disturbance (fig. III.5.2 d).

Discussion

It is known that WPW may either simulate AMI or mask the electrocardiographic abnormalities of AMI (Fineschi et al, 2004).

The first possibility is that of WPW mimicking AMI. WPW was confirmed in our patient by the presence of delta waves in leads V2 and V3. Probably, in addition to activation of the ventricles from the normal bundle branches, this patient presented activation of an accessory posteroseptal bypass tract. This resulted in negative delta waves in the inferior leads, which are morphologically identical to infarction Q waves and are present in 16% of patients with WPW (Lustik et al, 1999). Confusing the negative delta waves of WPW with a myocardial infarction is a common error. It is not possible to reliably differentiate negative delta waves from myocardial infarction based solely on EKG. If there is doubt whether the inferior Q waves represent infarction or negative delta waves, an echocardiogram may be needed to study inferior wall motion. The sensitivity of an echocardiogram is sufficient to rule out old inferior myocardial infarction if no wall motion abnormalities are seen (Yusuf, 1994).

If the inferior wall is hypokinetic or akinetic, further testing is indicated to determine myocardium at risk. But, in patients with preexcitation, a regular exercise stress test is not indicated (Pappone et al, 2012), as are sport activities. In our patient, chest pain was most likely caused by exercise tachycardia-induced ischemia. He had an exertional angina consistent with coronary artery spasm.

The second possibility is that WPW may also mask the EKG abnormalities of acute myocardial infarction. It is difficult to recognize acute myocardial infarction in patients with WPW because preexcitation masks the Q waves of transmural infarction (Mark et al, 2009; Kim and Knight, 2017).

Histologically, in our case the lesions at any level of the coronary system were absent, even in the presence of a myocardial infarction. This silent or almost-silent infarct occurred approximately 10 hours before the terminal episode, not impeding the athlete from habitual, vigorous physical training.

Referring to the relation between myocardial damage and myocell function, 3 types of cell deaths are described, because the damaged myocardial cell may stop functioning in irreversible relaxation or contraction, or may progressively lose its force and velocity (Baroldi & Silver, 1995; Centurion, 2011).

The first damage, known as infarct necrosis, which is an atonic cardiomyocyte death in irreversible relaxation, was associated in our study with others lesions. Focally, we saw an area of hemorrhagic infarct necrosis, usually seen after fibrinolytic therapy (Moreno et al, 2002), representing the reperfusion hallmark of ischemic myocardium. Rarely, a myocardial infarct may be hemorrhagic, such as in the case when associated with wall rupture (Yip et al, 2002),

or therapeutic procedures. Another observation concerns "wavy fibers", which are curly myocardial fibers representing an early sign of myocardial ischemia. Due to their lack of specificity, they do not allow a diagnosis of ischemia. In practice, wavyness of normal myocells is usually observed around hypercontracted myocardial fibers (Milroy & Parai, 2011).

Secondly, we revealed contraction band necrosis (CBN) lesions, representing a form of tetanic death in irreversible contraction. This form of myocardial necrosis has a morphofunctional pattern opposite to infarct necrosis. Here, the myocell is unable to relax and its function arrests in hypercontraction because of extreme reduction in sarcomere length. We observed several different CBN morphologies corresponding to different CBN histological ages.The lesion was either paradiscal, when limited to sarcomeres adjacent to the disc, or pancellular, with involvement of the entire myocell. The lesion healed by the replacement of damage cells with interstitial fibrosis (Oliva & Pascali, 2010). This injury has also been defined as coagulative myocytolysis. Coagulative is added to the term myocytolysis for emphasizing the coagulation of discal contractile proteins seen in a pancellular vacuolated CMs. Generally, CBN is associated with catecholamine infusion, detected in many other pathologic conditions, such as ischemic heart disease, electric shock, psychological stress, etc. (Sethi et al, 2007). Indeed, our patient was resuscitated with no success.

Coagulative myocytolysis must be differentiated from colliquative myocytolisis, which defines failing death of myocells by progressive function loss, which is a reversible lesion unlike CBN (Fineschi et al, 2005 2004). No colliquative myocytolysis lesions were found. No CM apoptosis was found, as well. Instead, interstitial fibrotic foci were noticed. Spotty fibrotic myocardium raised the question of ischemic lesion age. Are the ischemic events recent or repetitive? We believe that many other previous silent events developed before this last one and are demonstrated by different degrees of fibrotic interstitium. Indeed, localized myocardial fibrosis may be interpreted as a consequence of repetitive acute, nonfatal, events which, associated with chronic ischemia, lead to progressive myocardial fibrosis.

The myocardial disarray might be included among the adrenergic-related effects. Its linkage with catecholamine was already demonstrated in the literature (Fineschi et al, 2005). In our experience "pathologic" disarray occurred in conditions of adrenergic stress. At present, we have few human morphological evidence of adrenergic stress associated with unexpected death. Nevertheless, it is clear that CBN is observed in cases of sudden death (Gawaz, 2004). CBN was described in young subjects dying suddenly and unexpectedly with no clinical history of any disease. They had normal coronary arteries; the unique findings were CBN and myofiber breakup. The latter may be also associated with cardiac arrhythmia. We also consider most of these lesions related to adrenergic storm producing acute structural changes in cardiac tissue. Indeed, cardiopulmonary resuscitation per se, including noradrenalin infusion, electrical defibrillation, could explain many found lesions.

Final remarks

In patients with WPW syndrome, atrial fibrillation with a very rapid ventricular rate may be life threatening. Early recognition and correct treatment allows rapid restoration of normal sinus rhythm and may decrease morbidity and mortality.

III.6. Primary cardiac malignancies

III.6.1. Introduction

Primary malignant cardiac tumors are rare, regardless of the age group. The pathophysiology of cardiac tumors is heterogeneous and depends on the type of tumor (Neragi-Miandoab et al, 2007). Heart tumors are divided into two groups: (A)-primary heart tumors deriving from the heart; (B)-secondary heart tumors representing metastatic malignancies of other organs. The primary heart tumors may be benign (about 75%) or malignant (25%). From malignant types, sarcomas are the most common tumors, although other tumor types have been reported. When a cardiac tumor is discovered, it requires a rapid and full evaluation to characterize its nature so that an optimal management plan can be decided upon as soon as possible (Hamidi et al, 2010).

Virtually all types of sarcomas have been reported in the heart (Lam et al, 1993). Cardiac sarcomas are extremely rare, and for most types, only isolated case reports have been described (Kim et al, 2011).

Cardiac tumors may be symptomatic or found incidentally during evaluation for a seemingly unrelated problem or physical finding. The clinical manifestations largely depend on the size of the tumor, its localization, mobility and the degree of malignancy (infiltration, mass effect). Specific signs and symptoms generally are determined by the location of the tumor in the heart and not by histopathology (Burke, 1992).

The most common symptoms are: (a) – shortness of breath (including paroxysmal nocturnal dyspnea); (b) – impaired contractility, arrhythmias, heart block, or pericardial effusion with or without tamponade due to direct invasion of the myocardium; (c) – valve regurgitation or stenosis; (d) – embolic events; (f) – systemic features of inflammation; (g) – obstruction of the coronary arteries, producing symptoms of angina, myocardial infarction, heart failure (Simpson et all, 2008).

Mechanisms by which cardiac tumors may cause symptoms include: (a) – obstruction of the circulation through the heart or heart valves, producing symptoms of heart failure; (b) – interference with the heart valves, causing regurgitation; (c) – direct invasion of the myocardium, resulting in impaired contractility, arrhythmias, heart block, or pericardial effusion with or without tamponade; (d) – invasion of the adjacent lung may cause pulmonary symptoms and may mimic bronchogenic carcinoma; (e) – embolization, which is usually systemic but can be pulmonic, too; (f) – constitutional or systemic symptoms (Salcedo et al, 1992).

Taking into account the likelihood of cardiac tumors, the diagnostic procedures should be performed in all patients with unexplained cardiac murmurs, congestive heart failure, arrhythmias, which are accompanied by fever, anemia and weight loss of unknown cause. In such cases the basic laboratory tests should include tumor markers. Cardiologic diagnostic procedures should be widened with particular emphasis on imaging methods. Transvenous biopsy is a recommended diagnostic tool. (Hamidi et al, 2010).

In general, sarcomas proliferate rapidly and cause death through widespread infiltration of the myocardium, obstruction of blood flow through the heart, and/or distant metastases. Although complete resection is the treatment of choice, most patients develop recurrent disease and die of their malignancy even if their tumor can be completely resected (Kosuga et al, 2002). The median survival is typically 6 to 12 months, although long-term survival has been

reported with complete resection. Adjuvant chemotherapy has been used in an effort to improve on the poor results with resection alone. Patients with low-grade sarcomas may have a better prognosis (Bakaeen et al, 2009). A less aggressive course seems related to left atrium location, a low histologic grading with scarce cellular pleomorphism and low mitotic activity, absence of necrosis, myxoid tumor appearance, and absence of metastasis at diagnosis (Deyrup et al, 2006).

The most frequently described sarcomas include: angiosarcomas, rhabdo-myosarcomas, fibrosarcomas and leimyosarcomas (Putman et al, 1991).

Angiosarcomas (AS) are composed of malignant cells that form vascular channels. Angiosarcomas arise predominantly in the right atrium (Burke et al, 1992). ASs are the most common histological subtype, typically affecting middle-aged men. These tumors have a predilection for the right chambers, particularly for the right atrium. Echocardiography shows, a large, broad based mass near the inferior vena cava, extending intracavitary and into the pericardium, occasionally invading the caval veins or tricuspid valve (Lisy, 2007).

Rhabdomyosarcomas (RMS) constitute as many as 20 percent of all primary cardiac sarcomas. These tumors are most commonly found in adults, although they have also been described in children. Multiple sites of myocardial involvement are common, and there is no predominant location within any area of the heart. RMS**s** grow rapidly and are invasive, often extending to the pericardium before diagnosis (Castorino et al, 2000).

Fibrosarcomas (FMSs) are white fleshy ("fish flesh") tumors that are composed of spindle cells, and may have extensive areas of necrosis and hemorrhage. These tumors tend to extensively infiltrate the myocardium (Burke et al, 1992).

Leimyosarcomas (LMS) are spindle-cell, high-grade tumors that arise more frequently in the left atrium. These sarcomas have both a high rate of local recurrence and systemic spread (Pins et al, 1999).

All sarcomas of the heart are highly malignant cancers that tend to spread rapidly throughout the body. If they are diagnosed early enough, complete resection is necessary. However, most of these tumors will have metastasized before they can be diagnosed. Chemotherapy has not been particularly successful.

Other sarcomas, including liposarcoma, osteosarcoma, myxosarcoma and undifferentiated sarcoma occur extremely rare as primary malignancies of the heart. They usually arise in the left atrium and are highly invasive and aggressive (Simpson et al, 2008).

Objectives

There are very few studies assessing the results of cardiac surgery in individual centers. We present our experience in diagnosis of CTs, in an attempt to characterize and better understand disease presentation, location, treatment modalities, and overall survival of patients with CSs.

III.6.2. Cardiac sarcoma

Case presentation

Case 1

A 36-year-old woman came to our hospital with radiographic and echocardiographic examinations that revealed a right ventricular tumor that was spreading in the interventricular septum and right ventricular wall. The tumor was surgically approached by medial sternotomy,

but complete tumor resection was not possible because of the extensive tumor infiltration. The palliative intervention, consisting in partial excision of the tumor mass, aimed at releasing the right ventricular outflow tract.

Macroscopically, the tumor corresponded to a firm polypoid mass (fig. III.6.2.1.a), with large endocardial base, infiltrating the ventricular septum and projecting into the right ventricular cavity. The bulky tumor compressed the pulmonary valve, occluding the pulmonary artery orifice as well. The tumor had variegated appearance due to the presence of areas of necrosis and hemorrhage.

Histological examination of the tumor revealed a high grade cardiac sarcoma, without evidence of specific lines of differentiation. Microscopic study showed a heterogeneous hypercellular tumor, consisting of spindle cells, interspersed focally with multinucleated giant cells. The dominant histological feature of the tumor was marked cellular pleomorphism and increased mitotic activity (fig. III.6.2.1.b).The tumor had embolic features (fig. III.6.2.1.c). Immunohistochemical evaluation (fig.III.6.2.1.d) revealed a positive immunoreactivity to vimentin, suggesting a fibroblastic origin, and negative immunoreactivity to desmin and mesothelial markers, excluding a smooth muscle and mesothelial origin. These findings were suggestive of pleomorphic undifferentiated cardiac sarcoma.

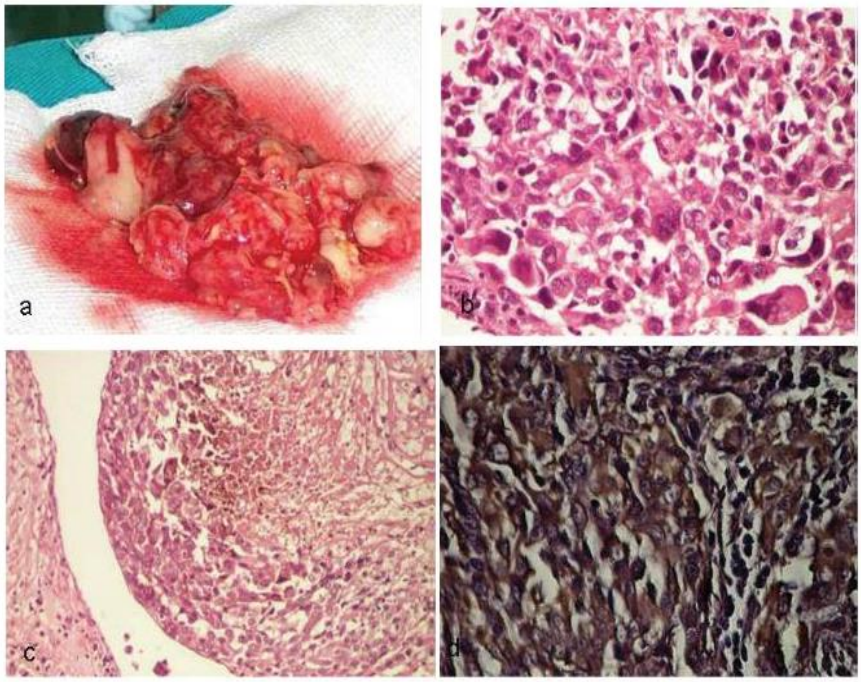

Figure III.6.2.1. Cardiac fibrosarcoma: a - Gross polypoid tumoral appearance; b - Pleomorphic tumoral cells and multinucleated giant cells (HE, 40x); c - Right ventricle: intracavitary tumoral embolus (HE, 20x); d - Focal positive immunoreactivity for vimentin in tumoral cells, 40x.

An important feature of the tumor was the tendency to pulmonary thromboembolism, supported by the presence of tiny tumor nodules (fig. III.6.2.1.c) dislodged from the right tumor mass without evidence of metastases. The thrombotic status was explained by the hypercoagulate status associated to cardiac tumor.

Regardless of histology, malignant cardiac tumors have a poor prognosis when tumor resection is difficult, they are undifferentiated, have areas of necrosis, and high mitotic index.

In this case adjuvant chemotherapy has a limited value, and radiation therapy is restricted by adverse cardiac effects.

Case 2

A 58-year-old male presented to our hospital for cardiac assessment. The patient had a 4-year history of recurrent pericarditis of unknown etiology, which responded to prednisone administration. At presentation he complained of progressive exertion dyspnea.

The clinical picture was normal except for shortness of breath. Blood reports were normal, except a mild anemia. Initial ECG and chest X-ray were unremarkable but, later, a second review of chest X-ray raised the suspicion of mild pericardial effusion. On transthoracic echocardiography, a lobulated mass of 3x2 cm in size, attached to the interatrial septum was evidenced. Global systolic function was preserved with an ejection fraction above 60%. Small pleural and pericardial effusions were observed. These findings were suggestive of right atrial myxoma. No metastases were seen. Coronary angiography showed a double source of tumor vascularisation: from LCX and RCA. The likelihood of an angiosarcoma was high.

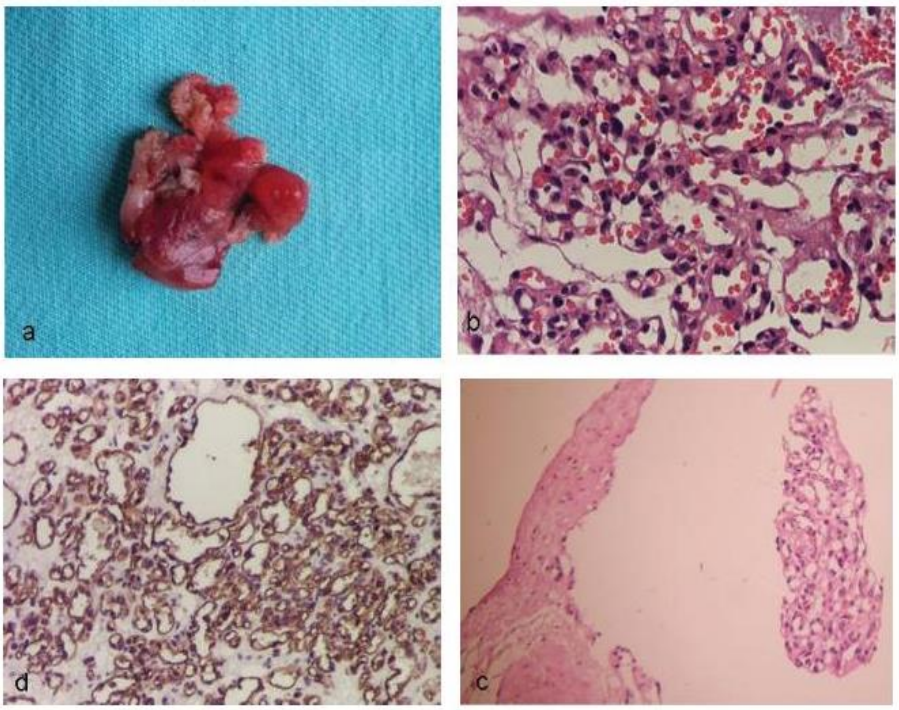

Figure III.6.2.2. Cardiac angiosarcoma: a - Gross appearance of a solid, lobulated, pinkish, tumoral mass with hemorrhagic foci; b – Pleomorphic tumoral cells form vascular structures containing erithrocytes (HE, 40x); c - Right atrium: intracavitary tumoral embolus (HE, 10x); d - Diffuse positive immunoreactivity for CD34 in tumoral cells, 20x.

The intraoperative findings showed a mobile, solid, gray-pinkish, tumor mass of moderate consistency, attached to the atrial septum on a large base (fig, III.6.2.2.a).

The tricuspid orifice and leaflets were morphologically normal. Histology (fig, III.6.2.2.b) revealed a low-grade angiosarcoma, with a low mitotic index and nuclear pleomorphism, infiltrating locally the tumor base.

Necrotic foci and hemorrhagic infiltration were focal tumor features. Tumor emboli detachment was also seen (fig, III.6.2.2.c). Immunohistochemistry confirmed that tumor cells were positive for CD34 and CD31 vascular markers (fig, III.6.2.2.d).

The postoperative transthoracic echocardiography showed normal sized and free cavities, left ventricle with normal global systolic function, pericardium without fluid, and intact intraatrial septum. Abdominal ultrasound was normal. The early course was uneventful and the patient was discharged from hospital in good condition, CT exams and cancer treatment being recommended.

Discussion

Primary cardiac neoplasms are extremely rare, with an autopsy prevalence of 0.001-0.28%. In adults, about 25% of primary tumors of the heart are malignant; angiosarcoma accounts for 35% to 40% of them, being the most common primary cardiac malignant tumor, followed by fibrosarcoma, representing 33% of cardiac sarcomas (Neragi-Miandoab et al, 2007). Because they are so rare, often they are missed or misdiagnosed.

The diagnosis of cardiac sarcoma is complicated by the fact that most cardiac tumors cause a variety of nonspecific clinical manifestations. The diagnosis of cardiac sarcoma is often not made preoperatively or even antemortem. It is overlooked because of the rarity of the lesion and the nonspecific nature of signs and symptoms (Simpson et al, 2008). Bloody pericardial effusion may cause cardiac failure. Myocardial involvement may lead to heart failure, as well. Endomyocardial masses cause valvular obstruction or insufficiency. Tumor fragments may embolize from the right side of the heart to the lungs and cause dyspnea. Local extension of the tumors may cause signs and symptoms such as superior vena cava syndrome (Zhang et al, 2008).

So, the diagnosis was delayed, being suggested, in the first case, by the markedly increase of tumor mass with obstruction of pulmonary ejection tract, and, in the second case, by recurrent pericardial effusions of unknown cause.

Imaging studies are an important tool in preoperative tumor diagnosis. Nowadays, advances in diagnostic techniques have facilitated accurate, noninvasive assessment of cardiac sarcomas (Mayer et al, 2007; Yuan et al, 2009). In cardiac tumors, the challenge is not only to differentiate between primary or secondary, and malignant or benign tumors, but also between neoplastic and nonneoplastic lesions. Imaging modalities are required for rapid diagnosis and staging, as well. Transthoracic echocardiography is the preferred diagnostic procedure for noninvasive imaging of cardiac tumors (Randhawa et al, 2011; Fussen et al, 2011). Preoperative investigations did not reveal any metastases in any of the cases. A diagnosis of cardiac tumor was made postoperatively by morphological examination in both cases. Although endomyocardial biopsy provides tissue to use in diagnosis, this biopsy is not absolutely necessary preoperatively because cardiac tissue is obtained during surgical exploration (Orlandi et al, 2010; Fletcher et al, 2006; Mayer et al, 2007). Microscopic examination performed on pieces of surgical excision was essential in the diagnosis, revealing in the first case an undifferentiated sarcoma and in the second case a pleomorphic differentiated angiosarcoma. A common histological feature in both our cases was the tendency of embolization, demonstrated histologically. Fibrosarcomas have an infiltrative growth pattern.

No cardiac chamber predilection has been noted. However, cardiac valvular involvement is found in as many as 50% of lesions (Mayer et al, 2007).

In the case one, we showed a pulmonary orifice occlusion due to pulmonary ejection tract involvement. Nearly 80% of cardiac angiosarcomas arise as mural masses in the right atrium (Fletcher et al, 2006; Mayer et al, 2007), especially the free wall, the interatrial septum being a rare location. Typically, they completely replace the atrial wall and fill the entire cardiac chamber. They may invade adjacent structures (e.g., vena cava, tricuspid valve).

In the case two, the tumor invaded the venous sinus impeding venous returning. The coronary venous sinus involvement was a cause of pericardial effusion by venous engorgement, without effects on venous returning due to the presence of an adequate collateral circulation with anterior cardiac veins and thebesian veins.

The prognosis of malignant cardiac tumors is generally the same: almost all cardiac sarcomas are rapidly fatal. It is suggested that age, gender, presence of differentiation, and histologic type do not affect prognosis (Mayer et al, 2007). A low level of mitotic activity and any therapy were the only significant factors affecting survival rate. Although the prognosis in patients with cardiac sarcomas is dismal, histologic grading is useful in predicting outcome, as has been shown for soft tissue sarcomas of other sites (Orlandi et al, 2010).

In pleomorphic undifferentiated fibrosarcoma, statistics showed that the average survival is of 6 months since diagnosis (Mayer et al, 2007; Orlandi et al, 2010). It is noted that undifferentiated cardiac sarcomas and non- removable malignant tumors have a poor prognosis (Mayer et al, 2007). The presence of tumor necrosis and high mitotic rate are also indicative of poor prognosis. It is reported a higher survival in relation to applied therapeutic modality (Orlandi et al, 2010).

In the first case, the partial tumor removal was correlated with an immediate good course reflected in the improvement of cardiac function due to releasing of the pulmonary outflow tract. However, further development was unfavorable, with only a 3-month post-surgical survival due to progressive deterioration of cardiac function by progressive local recurrent tumor invasion. Since admission, the patient was clinically *in extremis*, with progressive ventricular dysfunction.

Generally, angiosarcoma is characterized by a short clinical course, given its aggressive behavior, and delayed diagnosis, due to the non-specific clinical picture and its rarity (Zhang et al, 2008; Fletcher et al, 2006). It is appreciated that the average survival from initial symptoms to death is less than 9 months, and the features associated with longer survival include: left-sided tumor, complete tumor resection, post-operative adjuvant therapy, tumors with less than 10 mitoses/ HPF, and absent necrosis (Zhang et al, 2008).

In the second case, although the tumor was completely removed and well differentiated, the tendency to metastasis observed histologically made it a poor prognosis tumor, the patient dying in less than 5 months by lung metastases.

The usual scenario of progressive cardiac sarcomas is rapid metastases, tamponade, and cardiac heart failure. These most frequent causes of death are a good illustration for the therapeutic dilemma faced with this neoplasia.

So, what is the management of these life threatening tumors? The question of a new therapeutic strategy combining surgery with radiotherapy and chemotherapy is under debate. Complete or partial excision of primary or metastatic cardiac sarcoma can provide

hemodynamic improvement and relief from congestive heart failure. Postsurgical adjuvant radiation and chemotherapy have not proven consistently beneficial (Matebele et al, 2010; Penel et al, 2008). However, they can be beneficial in improving the symptoms and quality of life. The role of orthotopic heart transplantation for malignant cardiac tumors continues to be discussed.

Final remarks

These tumors are rare, data are scarce, mostly representing case reports and experiences of single institutions. Therefore, individual approach to every case is very important and treatment options should be discussed thoroughly in multidisciplinary teams.

SECTION II. Scientific carrier and future research developments

Plans for development of scientific career and future research

Scientific research is essential for the development of a scholar's teaching career.

A scientific career requires a permanent passion towards research, and the fields of research in which I have been involved so far will remain my priority for the future.

Therefore, one of my main goals is to pursue my research in order to identify new issues and, hence, obtain results that could lay the foundation of new interdisciplinary scientific papers. Development of interdisciplinary cooperation at the "Grigore T. Popa" University of Medicine and Pharmacy in Iași and with other medical schools in Romania and abroad.

There are two main domains of interest from my entire career focused on atherosclerosis and cardiac pathology diagnosis on EMB.

Atherosclerosis research

Coronary artery disease (CAD) continues to be the main cause of morbidity and mortality worldwide, in spite of the many ongoing advances in the last years in prevention programs, and pharmacological and myocardial revascularization treatments.

So far, my scientific work was focused on extending ATS pathology research from muscular medium size vessels (femoral, carotid arteries, etc) to large elastic arteries (aorta), In particular, in the light of my previous achievements and based on the significant expertise that I encountered in the field of atherosclerosis - oriented research my plan for further scientific development includes many directions of research.

One significant target is to highlight the importance of smooth muscle cell proliferation in the formation of atherosclerotic lesions in relation with plaque inflammation, which is involved in smooth muscle cell activation and proliferation.

Of particular interest in research is the in-depth study of inflammation related to atherosclerotic disease progression. Inflammation is a central driver of atherosclerosis and also a therapeutic target in atherosclerotic disease. The first clues about the role of inflammation in atherosclerosis came from the pioneering work of Dr. Russel Ross (Ross, 1999), who highlighted this pathogenic mechanism in response to endothelial damage that triggers local chronic inflammation. Additional research has shown that inflammation is an important factor in disease progression and plaque rupture, too. Inflammation, as a therapeutic target in atherosclerosis, reveals the need to focus research on the atherosclerotic inflammatory process, in the hope of finding a remedy that will control the evolution of the disease.

If subsequent studies indicated that in the progression of the plaque, the deep fissures and ulcerations of the plaque are a cause of luminal thrombosis, we are interested in establishing the roll of superficial erosion and eruptive calcified nodules, which are less frequent, but still important, in thrombi formation. The study is based on new morphological ATS classification, which took into account the three etiologies (rupture, erosion and calcified nodule) which lead to coronary thrombosis.

With advances in biomarkers and imaging of the atherosclerotic plaque, the concept of vulnerable plaque, as the precursor of plaque rupture, is still studied. Precursor lesions of plaque rupture, originally known as vulnerable plaques, were classified as thin cap fibroatheromas (TCFA). In our research we applied morphometry for assessing intimal thickness index (ITI), as a morphological hallmark of vulnerable plaques in vulnerable patients. Also, additional work is nedeed to identify arterial repair in the presence of plaques, with silent episodic thrombosis (healed plaque rupture and plaque fissures). In the future, we intend to apply this new classification for analyzing plaque progression and assessing the severity of ATS plaque by calculating a histological risk score.

The link between inflammation and atherosclerosis will be further studied taking into account epicardial adipose tissue enlargement. In the recent years, many publications have described the role of epicardial adipose tissue as a depot of inflammatory mediators. It seems that epicardial adipose tissue represents an active organ, releasing inflammatory factors in the systemic circulation. In order to elucidate this hypothesis, I started and planned to develop a research project for demonstrating that both epicardial adipose tissue and inflammation are a major player in cardiovascular diseases.

New advances in CAD diagnosis and pharmacological treatment, as well as percutaneous and surgical interventions have significantly influenced the disease progression. Therefore another area of research is the extending ATS pathology study from native vessels to atherosclerosis in saphenous vein grafts and neoatherosclerosis related by percutaneous coronary intervention with stents (bare, or non-medicated stents, BMS) or drug-eluting stents (DES).

In addition to the atherosclerosis of the native vessels, studied so far, which consists in the development of atherosclerosis in a natural environment, the two new types of atherosclerotic lesions are related to the formation of atheroma in an iatrogenic environment. One is saphenous vein graft atherosclerosis and the other is neoatherosclerosis in segments previously treated with stents.

We already started the analysis of the presence of atherosclerosis in saphenous veins, before their use as grafts in CABG surgery. But more researches are needed for analysing their progresion.

It would be of great interest in the future to study the neoatherosclerosis related to percutaneous coronary intervention with stents (bare, or non-medicated stents, BMS) or drug-eluting stents (DES). Currently, there is great interest in the neoatherosclerotic process as the cause of stent thrombosis, and its possible relationship with underlying native atherosclerosis. Neoatherosclerosis researches will highlight the deficiencies in secondary prevention strategies.

In conclusion, the comprehension of the new iatrogenic atherosclerotic processes, and the morphological comparisons of atherosclerotic plaque progression, should help advance understanding of native atherosclerotic disease, saphenous vein graft atherosclerosis, and neoatherosclerosis.

This, in turn, should aid the development of improved strategies for modifying cardiovascular risk and improving secondary disease prevention. The near future is aimed at generating ideas and new strategies for identifying and treating atherosclerosis in all its forms,

innovating anti-atherosclerotic treatment platforms, and strengthening secondary prevention programs.

Cardiac pathology research on endomyocardial biopsy method

Another major interest is the extended application of endomyocardial biopsy (EMB), not only as a useful diagnostic method, but also as a clinical research tool. Although cardiac imaging advances have significantly improved the noninvasive diagnosis of heart disease, it often remains a role for EMB as well. The probability of a specific pathological diagnosis based on cardiac EMB varies depending on the heart disease. The addition of immunohistochemistry and PCR to standard histologic analysis has enhanced the sensitivity of EMB. Specific diagnoses where EMB may be useful in native hearts include myocarditis (viral, giant cell, eosinophilic), amyloidosis, sarcoidosis, ARVC, etc. EMB use will continue to increase in frequency, and technique complications are low when EMBs are performed at profile centers. So, endomyocardial biopsy (EMB) remains the gold standard mode of investigation for diagnosing of many primary and secondary cardiac conditions. The need for EMB exists because specific myocardial disorders that have unique prognoses and treatment are seldom diagnosed by noninvasive testing.

Myocyte disarray, an indicator for diagnosing hypertrophic cardiomyopathy, is a common finding in biopsies taken from the right ventricle apex and apical septum. Disarray is an expected finding in this location and should not be used to make a specific diagnosis of primary hypertrophic cardiomyopathy, but EMB remains useful when it involves IVS with the avoidance of the ventricular apex.

The EMB for a sarcoidosis diagnosis has a low yield, because cardiac sarcoidosis tends to involve the base of the heart, which is not the area biopsied in the usual right ventricle endomyocardial biopsy procedure. We had good results on small basal IVS fragment obtained during mitral valve surgery in a patient with mitral stenosis.

The utility of a biopsy for diagnosis of arrhythmogenic cardiomyopathy depends on where the biopsy is taken. Free-wall and infundibulum biopsies would have a higher utility than those from the ventricular septum. With ventricular wall thinning and fatty infiltration, such a procedure may be considered too risky for free-wall perforation. Noninvasive evaluation may be more prudent in such a patient. But, targeting the interventricular septum in the area of the "triangle of dysplasia", EMB could be successful.

The EMB is useful for differentiating chronic cardiomyopathy changes from myocarditis in the setting of an episode of acute heart failure. Many such patients may actually have an acute exacerbation of a chronic condition. Biopsy may demonstrate chronic cardiomyopathic changes with fibrosis and hypertrophy. Although not always specific for a type of cardiomyopathy, such a finding has implications for patient prognosis, reversibility, likelihood of recovery and, perhaps, a treatment plan. If the patient has myocarditis, giant cell myocarditis can produce a severe acute clinical picture and may be successfully treated by immunosuppression.

Despite advances in the diagnosis and treatment of patients with cardiomyopathy, the prognosis remains poor. The etiology of dilated cardiomyopathy is often unknown and is assumed to be "idiopathic" or "viral;" however, only 50% of dilated cardiomyopathies are idiopathic and a small proportion of patients with non-ischemic cardiomyopathy have ventricular dysfunction proven to be viral in origin. Determination of the etiology of a

cardiomyopathy is important because it influences not only prognosis, but also medical and surgical therapy. So, the myocardial biopsy is likely to become important in our attempts to unlock the mystery of heart muscle disorders.

In the past, the myocarditis exam had a negative result being difficult to distinguish which cases were associated with viral replication and which were autoimmune-associated. Nowadays, with developing molecular biology techniques, their distinction using biopsy tissue may be of importance to help decide whether antiviral or immunosuppressive therapy is indicated. EMB remain therefore of increasing interest for evaluating primary dilated cardiomyopathy, especially in cases thought to follow myocarditis.

We used endomyocardial biopsies for detection of myocardial fibrosis and had good results. Previously, the only methodology available to assess myocardial fibrosis was the histopathological assessment of tissue EMB. This methodology enables qualitative macroscopic assessment after Masson Trichrome staining and quantitative absolute assessment of the collagen volume fraction in tissue samples by quantitative morphometry with picrosirius red, which specifically stains fibrillar collagen under polarized light. Although this technique offers an absolute quantification of fibrosis in myocardial samples, it has 3 evident drawbacks: 1) invasive biopsies are required; 2) sampling errors restrict the accuracy of biopsy in the case of localized fibrosis; and 3) fibrotic involvement of the whole LV cannot be determined.

The EMB may be useful for the differentiation between restrictive cardiomyopathy versus constrictive pericarditis. If the hemodynamics or imaging studies are not clear, an endomyocardial biopsy may demonstrate a myocardial cause for restriction, such as amyloidosis or iron storage. In constrictive pericarditis, such a biopsy would be normal or the cardiomyocytes might show atrophy. From our experience, EMB is useful in cardiac amyloidosis diagnosis. Using a myocardial biopsy, amyloidosis may also be diagnosed and typed, with treatment implications. It is important to note that an amyloid may be deposited solely in the heart, so a negative extracardiac biopsy (such as a fat aspirate or rectal biopsy) does not rule out cardiac amyloidosis. Differentiating the amyloid type is also important, and may be done by the pathologist.

The EMB may also be used for diagnosis of iron overload in the myocardium, which is important because the patients can be treated, potentially decreasing their heart failure. Iron is not normally found in the cardiomyocyte; therefore, any myocyte iron deposit found by the pathologist is abnormal. If iron overload is sufficient to cause congestive heart failure, the biopsy should be informative.

Cardiac neoplasms may also be diagnosed by EMB. Both right- and left sided neoplasms may be biopsied, allowing diagnosis and treatment planning. Avoiding sternotomy in an unresectable tumour may be a humane option. We applied the EMB technique in advanced tumor diagnosis and showed that it remains a useful diagnostic tool.

We consider that it is important to maintain some level of clinical interest and expertise in this procedure and the specialized interpretation of the pathology. Pathologists need to have good communication and interaction with the clinicians to ensure the best interpretation of the material. As with many aspects of medicine, a team approach is optimal. Future clinical applications are promising and the current judicious use of this procedure remains an important contribution to the care of our patients.

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