Histopathologic Characterization of Lesions in Rheumatic Valvulopathy

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Abstract

Cardiac valvulopathies may be caused by acquired or congenital diseases and result in valvular stenosis and/or insufficiency. For a correct diagnostic evaluation of cardiac valves, and in particular of rheumatic valvulopathy, pathologists should know the patient's clinical history, the degree of insufficiency and/or stenosis and echographical data. Histopathologic features of rheumatic valvulopathy include precocious, tardy and terminal lesions. Rheumatic valvulopathy may produce mitral insufficiency. Mitral valve incompetence has been classified into three types by echography (Carpentier, 1980). 56 cases of valvular samples have been examinated at our institution from January 2004 to January 2008. Any case was processed according to diagnostic/operative protocol we prepared carefully: gross examination, photographic documentation, sampling, standard stains and histochemical special stains. On the basis of the operative diagnostic protocol, histopathologic diagnosis of rheumatic valvulopathy was effected in three cases. Special methods of histochemistry, in addition to hematoxylin and eosin, are fundamental for the differential diagnosis of valvulopathies. Histopathologic diagnosis of rheumatic valvulopathy is complex and requests several histomorphologic data: our operative diagnostic protocol is extremely useful to reach an anatomo-pathologic diagnosis of surgical sample, which constitutes an essential element to confirm preoperative clinical-echographic diagnosis.

Introduction

Normal mitral valve leaflets are composed of four layers. From the atrial to the ventricular versant, they are the auricularis, the spongiosa, the fibrosa, and the ventricularis. The auricularis is a thin layer containing collagen and elastic tissue and directly subjacent to the endotelium of the atrial surface. The spongiosa is formed mainly by delicate myxomatous connective tissue with abundance of star-shaped granules of proteoglycan materials. This layer contains connective tissue cells (fibroblasts, myofibroblasts, and poorly differentiated mesenchymal cells), small amounts of elastic fibers and collagen fibrils. The fibrosa forms the structural core of the leaflet and is composed of dense bundles of collagen, which extend from the annulus and are continuous with the chordae tendineae. The ventricularis is a thin layer composed of linearly arranged elastic fibers, which are continuous with the elastic layer of the chordae tendineae. The distal portions of the leaflet, until the free edge, are constituted only of fibrosa and spongiosa. Normal chordae tendineae are composed of two connective tissue layers: the large fibrous core composed of dense collagen bundles and a surrounding layer of elastic fibers. Cardiac valvulopathies may be caused by acquired or congenital diseases and result in valvular stenosis and/or insufficiency. For correct diagnostic evaluation of cardiac valves the pathologist should know the patient's clinical history and the degree of insufficiency and/or stenosis and echographical data. Some of these valvulopathies are listed briefly below [1, 2].

Usually mitral valve prolapse is an isolated cardiac lesion, but also it may be associated with Marfan syndrome, hypertrophic cardiomyopathy, ostium secundum atrial septal defect, Ehlers-Danlos syndrome, and Ebstein's tricuspid anomaly.

Mitral valve prolapse (Barlow's disease, floppy valve) is the most common valvular abnormality. Gross features characteristic of mitral valve prolapse are myxomatous thickening and redundancy of the valve leaflets particularly at the lines of closure, interchordal hooding, increase in leaflet length with formation of dome-like deformities. Also abnormal insertion, elongation and rupture of chordae may occur. The valve is typically glistening white, or whitish-blue with appearance of myxoid degeneration caused by excessive accumulation of proteoglycans in the spongiosa that results in

disruption of the fibrosa and expansion and elongation of valve leaflets. The atrial surface shows elastic fiber duplication and fibrosis. A precise diagnosis is not always possible, especially if only a portion of valve is resected. The differential diagnosis includes postrheumatic disease, which demonstrates commissural fusion, disorganized valve architecture, leaflet retraction, and chordal thickening and fusion. Rheumatic mitral stenosis is the most important clinical manifestation of chronic rheumatic heart disease; mixed stenosis and regurgitation is rare. Concurrent involvement of the aortic valve is common, often resulting in concomitant excision of the aortic valve. Characteristic pathologic findings are commissural fusion; retracted, fibrotic leaflets; thickened, fibrotic and shortened chordae tendineae; calcification may be severe, mild, or absent, and is most prominent in the commissures. Rheumatic valvulopathy will be extensively treated below [1-6].

The majority of cases of *infectious endocarditis* occurs in the setting of an anatomically abnormal valve or abnormal flow dynamics across valve or congenital shunts. Many cases, which occur on normal valves, arise in patients with predisposing noncardiac conditions, such as intravenous drug abuse, alcoholism, immunosuppression, and colon cancer. Grossly, there are friable vegetations (appearance varies from soft gray-pink to firm yellow-brown), that may be present anywhere on the valve surface, often attached to the line of closure. In cronic lesions, there may be focal leaflet fibrotic thickening, calcification, and perforation without residual vagetations. Histologically, the vegetation typically consists of platelets, fibrin, and acute and chronic inflammatory cells. The microorganisms are generally present in areas of inflammation, but may be difficult to identify them [1, 2].

Another valvular disease is observed in patients who had taken the anorectic drug combination fenfluramine-phentermine (fen-phen). Grossly, *fenfluramine-phentermine valvulopathy (S. fen-phen)* shows thickening of the valve leaflets with chordal fusion. Typical plaques are made up of myofibroblastic proliferation with a myxoid extracellular matrix and prominent cellularity of the endocardial surface. Small vascular channels and slight lymphocytic accumulations are often present, while the underlying valvular architecture is intact [7, 8].

Libman-Sacks endocarditis is rare, but it is seen in half of patients dead by *systemic lupus erythematosus* and may occurs on the tricuspid valve alone or together with mitral valve or with pulmonary valve and rarely on aortic valve. Vegetations are intermediate in size between those of rheumatic endocarditis and infectious endocarditis and they may be present anywhere on the valve surface, on chordae tendineae and on parietalis endocardium around. Vegetations consist of fibrin, cellular debris, degenerating valve tissue, and inflammatory cells; hematoxilin bodies (made by nuclear debris) may be present in the acute phase. Necrosis and endocardic ulcerations with trombotic material deposition result in verrucous vegetations [9].

Rheumatic cardiopathy Rheumatic disease is caused by A group hemolytic *Streptococcus* which induces cruciate immunity between bacterial antigens and tissue antigens (i.e. connective tissue, endocardial glycoproteins, myocardial muscular tissue sarcolemma, cardial myosin, etc.). Rheumatic disease may cause endocarditis, pericarditis and myocarditis. For the diagnosis of acute articular rheumatism, main diagnostic criteria are carditis, vagrant polyarthritis, chorea, erythema marginatum and subcutaneous nodules; minor criteria are arthralgiae, fever, and increased VES and/or PCR. In acute attack only myocarditis may cause patient death (incidence rate 1%). Myocardium is the cardiac tissue where development of typical rheumatic granuloma occurs [10]:

– I month: interstitial connective dissociation by basophil oedema and fibrinoid necrosis;

– II month: focal granuloma (Aschoff nodule), oviform, perivascular with central fibrinoid necrosis area, formed by Aschoff cells, Anitschkow cells, lymphocytes and plasmacells; Anitschkow cells are large histiocyte elements with large vesicular nucleus with central ribbonlike chromatin connected to cariolemma by thin ramification. In longitudinal nucleus section chromatin is millepede-like, in transverse nucleus section chromatin, in a clear halo, confers to nucleus an appearance of owl eye. Aschoff cells nuclear features are alike, but these cells are much voluminous, with large basophil cytoplasm, and generally they are multinucleate.

– After three months: there is sclero-cicatricial evolution with fibroblasts and many collagenous fibers instead of granuloma and small necrotic muscular areas.

Rheumatic myocarditis histological diagnosis may be get from auricle drawn during rheumatic endocarditis surgery or from endomyocardial biopsy. Auricle biopsy helps to identify rheumatic endocarditis in postinflammatory scarring, undistinguishable from other endocarditis with only histological endomiocardium examination and with Jones criteria inadeguate to diagnose the rheumatic disease.

Undeterminate acute cardiac decompensation post streptoangina, without distintive rheumatic disease symptomatology needs endomyocardial biopsy. Rheumatic endocarditis is a valvulopathy, also chordae tendineae and papillary muscles are involved; this is the rheumatic carditis manifestation which leaves greatest final results. Mitral and aortic valves are most frequently involved, while isolated right cardiac valves involvement is unusual [1, 2, 5, 6].

Materials and methods

56 valvular specimens have been examinated (28 cases from January 2004 to October 2006 and 28 cases from November 2006 to January 2008). In the 2004-2008 period a diagnostic/operative protocol and valvulopathy's diagnostic guidelines were carefully prepared by anatomic-pathological and clinical-echographical study. Any case has been processed according this protocol, that is explained below:

1) **Gross examination.** The gross examination is the most important aspect of the pathologic examination of valves [1, 2].

– Identification of the valve (right/left atrioventricular, aortic/pulmonary valve)
– Complete or partial valve (number of recognizable cuspides)
– Diameter or circumference of annulus fibrosus or size of partial valve
– Description of valvular surfaces and lesions (i.e. vegetations, erosions, ulcerations, commissurae fusion)
– Lesions topography (focal or diffuse, only on the valvular border or on all valvular surface, one or both versants)
– Description of chordae tendineae as normal, thickened or fused, thinned or elongated, and presence of rupture.
– Description of papillary muscles.

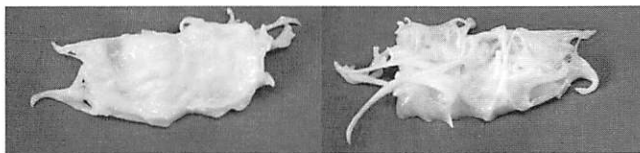2) **Photographic documentation** of both valvular versants (fig. 1).

Fig. 1 - Photographic documentation: atrial (left) and ventricular (right) versants.

3) **Sampling.** The specimen is fixed in neutral buffered formalin and sampled in toto (fig. 2) by consecutive sections from implantation basis to free border (with previous China ink labeling of implantation basis).
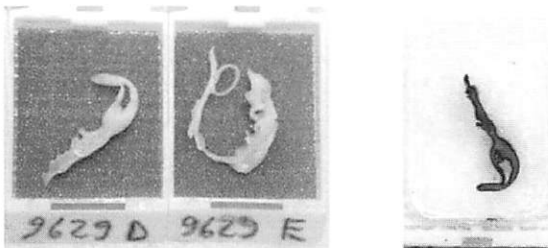

Fig. 2 - Sampling.

4) **Standard stains and histochemical special stains.**
Sampled material is embedded in paraffin blocks and 3 µm serial sections from each of them are cut and stained (fig. 3) with:
– Hematoxylin and eosin: histological standard stain.
– Alcian Blue-Van Gieson to point out acid mucopolysaccharides, collagen fibers and global histomorphological evaluation.
– Periodic Acid Schiff (PAS) to reveal neutral mucopolysaccharides.
– Weigert – Van Gieson for the examination of elastic and collagen fibers.
– Azan Mallory's trichrome stain for the global histomorphological evaluation.
– Congo red to detect eventual amyloid deposits [11].

Fig. 3 - Slides by protocol stains.

5) **Microscopic examination.** All the slides must be examined by optical microscopy and those Congo red stained also by polarized light microscopy, which shows green coloured amyloid (Fig. 4).

6) **Diagnosis** is possible only if the extension and the distribution of elementary lesions are evaluated, with clinical and echocardiographical data support.
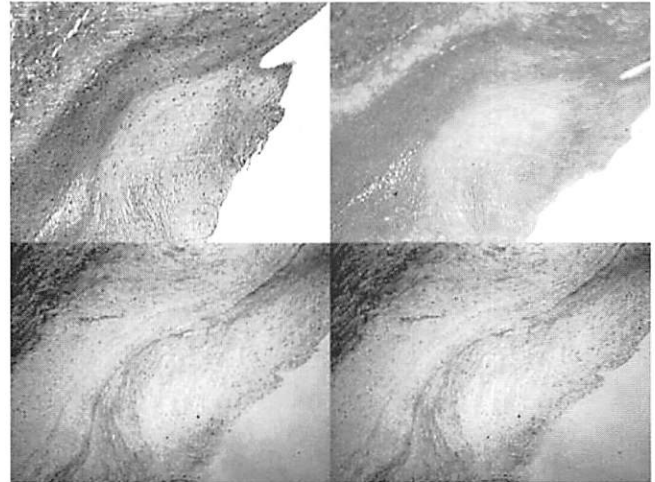

Fig. 4 - Examples of standard stains and histochemical special stains: hematoxylin and eosin (top, left), Alician Blue Van Gieson (top, right), Weigert-Van Gieson (bottom, left) and Azan-Mallory (bottom, right).

Results

On the basis of the operative diagnostic protocol, histopathologic diagnosis of rheumatic valvulopathy was made in three cases (Tab. 1). Special stains (e.g. Weigert-Van Gieson) allow to identify rheumatic versus myxomatous valves by characteristic locations of fibrous deposits: chronical rheumatic valve shows diffuse fibrosis of the leaflet with loss of distinction of the specific layers, while myxomatous valve shows reactive deposits of fibrous tissue upon the ventricular and atrial surfaces. The accumulation of proteoglycans and mucopolysaccharides is stained by Alcian Blue and Periodic Acid Schiff: in floppy valves it is observed mainly in the spongiosa and extended diffusely into other layers; in nonfloppy valves (e.g. rheumatic valves) it is in focal and limited areas in the spongiosa near the

Histopatological diagnosis	2004 - 2006		2004 - 2008	
	No./total	%	No./total	%
Acute endocarditis	-	-	1/56	1.7
Myxoid degeneration	9/28	32	32/56	57
Mixed degeneration with myxoid-fibroelastosic pattern	5/28	18	7/56	12.6
Postinflammatory valvulopathy	9/28	32	11/56	19.8
Rheumatic endocarditis	3/28	11	3/56	5.5
Libman-Sacks endocarditis	1/28	3.5	1/56	1.7
Fibroconnective degeneration by drugs (S. fen-phen)	1/28	3.5	1/56	1.7

Table 1 - Cases 2004 -2008

valvular annulus, in the ventricularis at the site of chordal insertion, in the auricolaris along the line of closure and the free edges.

Weigert-Van Gieson and Azan Mallory's trichrome also are important for the evaluation of elastic and collagen fibers and global histomorphological architecture. In fact, structural abnormalities in all connective tissue are demonstrated by these special stains. The elastic fibers in nonfloppy valves increase in the myxomatous areas with thin and smaller fibers than normal, but arranged in bundles. In floppy valves elastic fibers are disrupted and finely granular. Furthermore, collagen fibrils are arranged loosely and irregularly in floppy valves; in contrast, in the myxomatous areas of rheumatic or other nonfloppy valves they are arranged in small bundles [5, 11, 12] (Figs. 5 and 6).
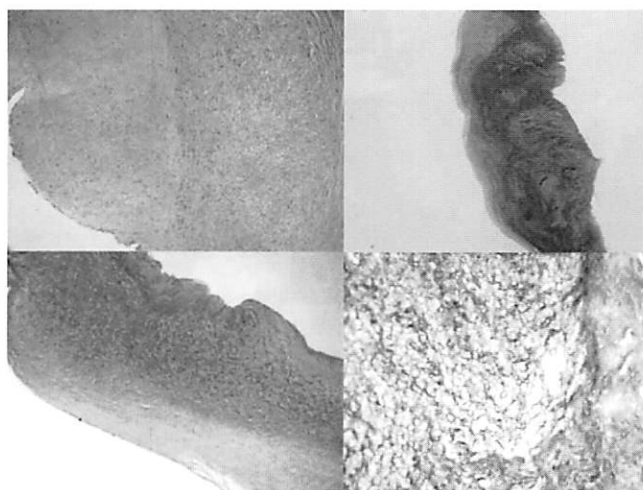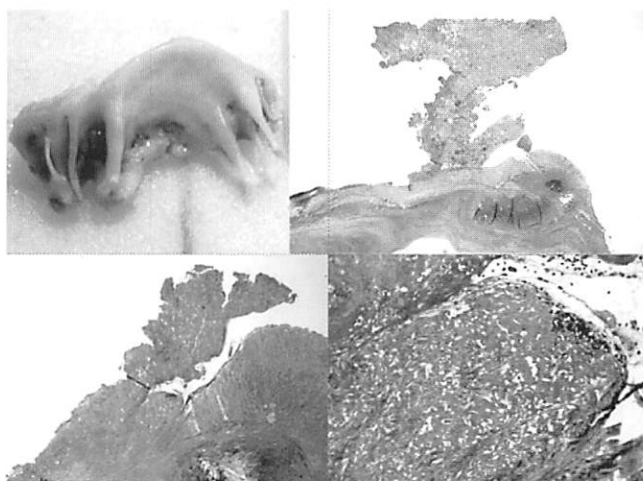

Fig. 5 - Myxoid degeneration (Barlow's disease)

Fig. 6 - Infectious endocarditis: fibrin vegetations

Histopathological features of rheumatic valvulopathy

Early lesions. The initial manifestation of serous valvulitis is diffuse valvular oedema. In early weeks of acute attack, fibrinoid necrosis and a diffuse inflammatory infiltrate of lymphocytes, rare fibroblasts and Anitschkow cells-like elements appear; Aschoff nodule, typical of the myocardium, is not seen in the endocardium. Small and

focal erosions may appear, mostly on occlusion rims and on chordae tendineae. Oedema and inflammatory infiltrate make tension in endocardium. Swollen valvolar connective may protrude through these solutions of continuity making small vegetations (13 mm), named verrucae, rosary disposed along occlusion rims and chordae tendineae, where fibrin and platelets are deposited. Verrucae do not tend to break off making emboli, because they are formed by valvular connective protruding from valvular surface.

Late stage lesions. Inflammatory lesions are replaced by overabundant connective tissue, rich in thick and glassy collagen and neoformed blood vessels with subversion of structure in tunicae of vela and pachynsis, stiffering and retraction of striked parts. Verrucae organize becoming compact and permanent structures and promote, together with overhanging thrombotic material, the fusion of commissurae. Recurrences, which are characteristic of this disease, renew acute alterations and store up their indelible results (Figs. 7 and 8).
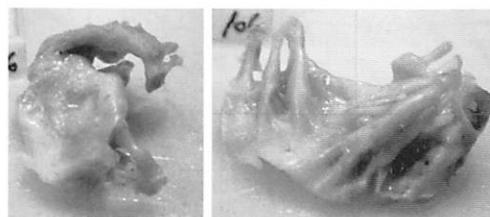

Fig. 7 - Gross features of rheumatic valvulopathy: atrial (left) and ventricular (right) versants.
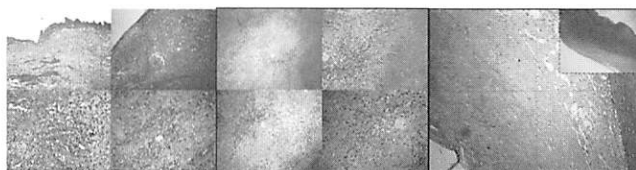

Fig. 8 - Histopathologic features of rheumatic valvulopathy: small verrucae (left), diffuse inflammatory infiltrate (center) and neoformed vessels (right).

End stage lesions. The terminal pattern of rheumatic endocarditis, called postinflammatory scarring, is fibrosis with plastic rearrangements often responsible for very important deformities. This pattern lost any gross and microscopic specific morphologic feature; therefore, if histological signs of rheumatic myocarditis or anamnestic information of previous rheumatic fever episode are absent, only a descriptive diagnosis should be made. In fact the same pattern may be caused by several diseases, e.g. *Brucella melitensis, Coxiella burnetii* infections, etc. [10]. Rheumatic valvulopathy may produce mitral insufficiency. Incompetent mitral valves have been classified into three
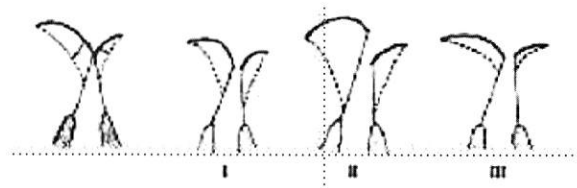

Fig. 9 - Carpentier's types of mitral insufficiency.

types by echography (Carpentier 1980, Fig. 9): type I: the free edges of the leaflets remain below the plane of the annulus during systole and open normally during diastole; this condition occurs when there is annulus dilatation or leaflet perforation (as in cardiomyopathy or in infective endocarditis); type II: the free edge of one or both leaflets goes beyond the plane of the annulus during systole (prolapse during systole), by elongation or rupture of chordae tendineae or of the papillary muscles (as in degenerative desease or in ischaemic disease); type III: restrictive movement during diastole with incomplete opening (IIIa) or during systole with incomplete closure (IIIb) of leaflets; these occur respectively in rheumatic disease with commissural fusion and leaflet or chordal thickening and in rheumatic, ischaemic or functional cardiomyopathy with chordal retraction, papillary muscle retraction or displacement [12-14].

The University Cardiosurgical team in Genoa performs conservative surgery instead of substitutive surgery with prothesis installation in type II (Tab. 2). The most common pattern of isolated mitral insufficiency (38-62%) is mitral valve prolapse distinct in primary or Barlow's disease (sporadic, familiar or associated with connectivopathies) and secondary (associated with rheumatic cardiopathy and/or infective endocarditis).

Histopathological diagnosis	No. /Total (%)	Age	Mitral insufficiency (types)			Surgical approach	
			I	II	III	CONS	SUBS
Myxoid degeneration	9/28 (32%)	58	0%	100%	0%	100%	0%
Mixed degeneration with myxoid-fibroelastosic pattern	5/28 (18%)	55	0%	100%	0%	80%	20%
Postinflammatory valvulopathy	9/28 (32%)	54	0%	77%	23%	77%	23%
Rheumatic endocarditis	3/28 (11%)	40	0%	0%	100%	0%	100%
Libman-Sacks endocarditis	1/28 (3.50%)	43	0%	0%	100%	0%	100%
Fibroconnective degeneration by drugs (S. fen-phen)	1/28 (3.50%)	38	0%	0%	100%	0%	100%

Table 2 - Cases 2004–2006: clinical-pathological data (CONS= conservative surgery; SUBS= substitutive surgery).

Discussion

Special histochemical methods, in addition to hematoxylin and eosin, are fundamental in the differential diagnosis of

valvulopathies. Histopathologic diagnosis of rheumatic valvulopathy is complex and requires several histomorphologic data: in this setting, in the period 2004-2008, an operative diagnostic protocol has been restated by our Department, and it resulted extremely useful to reach an anatomo-pathologic diagnosis on surgical samples, which constitute an essential element to confirm preoperative clinical-echographic diagnosis.

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