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Letter to the Editor

Epicardial adipose tissue has an increased thickness and is a source of inflammatory mediators in patients with calcific aortic stenosis☆

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It is widely recognized that biological processes leading to calcific aortic stenosis (AS) include chronic inflammation, lipoprotein deposition and activation of specific osteogenic and apoptotic signaling pathways [1]. These phenomena resemble those observed in the early stages of coronary artery disease (CAD). Further, AS and CAD share common risk factors, early pathogenesis, and clinical coexistence with a high percentage (40% to 75%) of patients with severe AS and concomitant CAD [2]. Thus, it is reasonable to hypothesize that the pathogenesis and progression of AS and CAD might be induced by similar stimuli, largely represented by the inflammatory activity intrinsic to the atherogenic process.

Epicardial adipose tissue (EAT) represents a visceral fat depot which plays several protective functions for the heart in physiologic conditions [3]. However, it is known that EAT may also play an unfavorable activity for the heart. In fact, it is a source of several pro-inflammatory and pro-atherogenic cytokines, which can biologically influence the myocardium and epicardial coronary arteries through paracrine or vasocrine actions [3]. It has been reported that EAT thickness represents a specific marker of visceral adiposity which is strongly associated with the presence and severity of atherosclerotic coronary artery disease (CAD) [4]. In this study, we sought to investigate whether EAT could also contribute to the inflammatory burden of AS. To this aim, we analyzed echocardiographic EAT thickness and EAT secretory profile obtained from patients with calcific AS.

We enrolled 95 patients with severe, isolated, calcific AS, referred to cardiac surgery for aortic valve (AV) replacement. Exclusion criteria were: 1) pathologic conditions already known to be associated with increased echocardiographic EAT thickness and/or altered inflammatory EAT status (CAD, metabolic syndrome, atrial fibrillation) [3,5]; and 2) chronic inflammatory diseases which could affect EAT thickness and/or systemic and local inflammatory profiles. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by our institution's human research committee. All patients provided written informed consent.

We measured EAT thickness by echocardiography in AS patients and in a control group of 44 healthy subjects matched for age, gender and body mass index (BMI). Intra and inter-observer reproducibility for echocardiographic EAT thickness assessment was excellent (0.962 and 0.951, respectively).

In patients referred to aortic valve replacement, plasma and EAT were collected and analyzed by human cytokine 27 multiplex immunoassay for the assessment of systemic and local inflammatory status. Table 1 illustrates demographic and clinical characteristics of the study population. EAT thickness was markedly increased in patients with AS with respect to controls (9.85 ± 2.78 vs 4.91 ± 1.27 mm; $p < 0.0001$). Demographic and clinical variables were also evaluated after study population stratification above and below EAT thickness median value (10 mm). There were no differences between the two groups for age, BMI, glomerular filtration rate, and atherosclerotic risk factors. Interestingly, as regard to cardiovascular therapy, only statin use was significantly higher ($p < 0.04$) in patients below the EAT thickness median value. No differences were found between the two groups in plasma inflammatory mediator levels, except for PDGF and VEGF. Contrarily, in EAT secretome, 22 of 27 measured inflammatory mediator levels were significantly higher in patients above the EAT thickness median value (Table 2). Further, EAT levels of several inflammatory cytokines, such as IL-6, IL-8, IL-1β, and TNF-α showed a highly significant increase

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Table 1
Demographic and clinical characteristics of the study population.

Age, years	72.52 ± 11.22
Gender Female, % (n)	60.0% (57)
BMI	27.66 ± 4.61
Dyslipidemia, % (n)	42.1% (40)
Diabetes, % (n)	29.5% (28)
Hypertension, % (n)	66.3% (63)
Smokers, % (n)	26.3% (25)
GFR < 60 ml/min	8.4% (8)
Medications	
Aspirin, % (n)	40.0% (38)
Statin, % (n)	37.9% (36)
ACE-I/ARBs, % (n)	53.7% (51)
BBs, % (n)	51.6% (49)
Echocardiographic characteristics	
LVEF, %	64.35 ± 12.33
E/A	3.16 ± 15.75
E Dec, msec	248.50 ± 87.77
E/e′	15.43 ± 5.96
AV area, cm^2	0.8 ± 0.2
Mean gradient, mm Hg	44.27 ± 19.66
Mass, gr	180.50 ± 45.02
Mass/BSA, gr/m^2	99.36 ± 27.66
RWT	0.44 ± 0.10
EAT thickness, mm	9.85 ± 2.78

BMI, body mass index; GFR, glomerular filtration rate; ACE-I, Angiotensin Converting Enzyme inhibitors; ARB, Angiotensin Receptor Blockers; BBs, Beta-blockers; LVEF, Left Ventricular Ejection Fraction; AV, aortic valve; BSA, Body Surface Area; RWT, Relative Wall Thickness; EAT, epicardial adipose tissue.

($p < 0.0001$) compared to systemic levels (Table 2). We also tested the relationship between EAT echocardiographic thickness and its inflammatory profile. Interestingly, we found a close direct correlation between EAT thickness and levels of secreted inflammatory mediators. In particular, EAT thickness significantly correlated with levels of IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-17, basic FGF, eotaxin, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF and TNF-α.

The relationship between EAT thickness and the presence and severity of atherosclerotic CAD has been well established [4]. Of note, our study provides the first evidence that EAT thickness is also increased in patients with isolated AS. This observation further supports previous evidence indicating that CAD and AS share common pathogenetic mechanisms. In this regard, histologic studies have demonstrated that, in the early AS disease, the biological processes show similar characteristics to those observed in the coronary atherosclerotic plaque [6].

The 'atherogenicity' of EAT is addressed to secretion of inflammatory cytokines, such as TNF-α, MCP-1, IL-6, IL-1β, plasminogen activator inhibitor-1 and resistin [7]. Interestingly, in CAD patients, local levels of these inflammatory mediators do not correlate with plasma concentrations of circulating cytokines and are independent of several clinical variables, such as obesity, and diabetes [7]. The present study evaluated the secretory characteristics of EAT secretome of patients with AS. We found that several inflammatory and pro-atherogenic mediators were significantly increased in EAT. In particular, IL-6 showed an enormous increase in EAT with respect to plasma. The involvement of IL-6 in AS has been recently described by El Husseini et al. [8]. These authors demonstrated that high expression of IL-6 promotes the mineralization process of AV leaflets, which is partially reversed by IL-6 inhibition. Further, it has been described that pro-inflammatory stimulation promotes the transition of AV interstitial cells toward inflammatory cells resulting in a significantly increased production of IL-6, IL-8 and MCP-1 [9]. As regard to IL-1β, we found a twelve fold increase of this cytokine in EAT secretome compared to systemic values. Histologic studies of stenotic AV leaflets have demonstrated increased levels of this potent pro-inflammatory cytokine which promotes matrix metalloproteinase expression and cell proliferation in calcific AV and has been implicated in the pathogenesis of several inflammatory diseases [9]. TNF-α, that showed 2.3 fold increase in EAT vs plasma in our study, has also been

Table 2
Plasma and EAT secretory profile of AS patients stratified according to EAT thickness median value.

	PLASMA			EAT		
	Pts below EAT thickness median value pg/ml	Pts above EAT thickness median value pg/ml	p value	Pts below EAT thickness median value pg/ml	Pts above EAT thickness median value pg/ml	p value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
PDGF	2212.1 ± 995.7	1107.0 ± 625.1	.010	34.1 ± 1.8	67.0 ± 32.8	.056
IL-1β	4.8 ± 2.2	4.8 ± 1.5	.963	47.8 ± 10.5	84.4 ± 6.3	.000
IL-1ra	525.1 ± 304.9	447.4 ± 242.3	.541	685.2 ± 137.6	1436.1 ± 163.6	.000
IL-2	30.2 ± 41.8	24.2 ± 38.1	.748	17.9 ± 1.6	81.4 ± 6.9	.000
IL-4	4.6 ± 1.7	3.5 ± 1.3	.129	2.5 ± 0.9	6.8 ± 1.0	.000
IL-5	3.5 ± 1.4	4.2 ± 2.5	.340	1.5 ± 0.5	3.3 ± 1.1	.010
IL-6	43.0 ± 34.8	27.6 ± 16.3	.255	52,114 ± 28,322.	112,394.1 ± 18,003.4	.004
IL-7	17.6 ± 8.1	18.0 ± 17.1	.938	2.7 ± 0.6	5.3 ± 0.8	.000
IL-8	61.6 ± 32.6	44.3 ± 14.1	.172	7222.9 ± 3785.8	134,151.5 ± 14,886.3	.000
IL-9	26.4 ± 13.4	28.4 ± 22.9	.792	6.3 ± 1.8	18.0 ± 1.8	.000
IL-10	50.3 ± 59.4	35.0 ± 29.4	.503	33.4 ± 3.0	229.8 ± 99.6	.002
IL-12	56.3 ± 47.4	41.7 ± 51.6	.504	32.6 ± 8.2	84.1 ± 48.7	.048
IL-13	15.2 ± 7.1	11.4 ± 7.9	.246	13.2 ± 3.0	12.7 ± 2.7	.792
IL-15	74.8 ± 27.6	132.3 ± 56.3	.093	322.6 ± 54.7	388.4 ± 50.5	.083
IL-17	110.5 ± 58.4	128.1 ± 128.6	.655	41.7 ± 15.3	240.7 ± 34.6	.000
Eotaxin	119.6 ± 44.9	124.0 ± 97.7	.888	26.0 ± 7.2	177.3 ± 15.6	.000
FGF basic	95.6 ± 33.1	135.6 ± 103.1	.176	205.7 ± 39.8	389.0 ± 104.7	.006
G-CSF	111.3 ± 46.3	131.8 ± 62.6	.382	3548.6 ± 615.5	85,329.2 ± 81,013.9	.054
GM-CSF	119.7 ± 83.5	124.6 ± 130.6	.913	612.5 ± 21.5	620.9 ± 103.6	.864
IFN-γ	159.2 ± 80.4	147.6 ± 38.4	.707	134.5 ± 18.4	371.6 ± 42.7	.000
IP-10	1635.3 ± 1125.0	1116.5 ± 603.5	.241	179.9 ± 140.5	3809.0 ± 2601.8	.014
MCP-1	152.4 ± 55.9	146.0 ± 117.3	.859	3709.8 ± 190.5	18,155.8 ± 2274	.000
MIP-1α	8.4 ± 6.5	9.8 ± 7.3	.646	30.7 ± 4.3	638.6 ± 122.3	.000
MIP-1β	226.9 ± 238.4	148.4 ± 58.0	.375	368.3 ± 93.7	1646.2 ± 174.7	.000
RANTES	26,332 ± 26,881	12,707.6 ± 10,742. 5	.187	233.4 ± 150.7	301.3 ± 98.2	.423
TNF-α	51.9 ± 23.4	80.8 ± 64.4	.129	63.9 ± 9.6	207.8 ± 17.6	.000
VEGF	187.6 ± 102.0	64.6 ± 44.4	.004	465.1 ± 198.3	1143.4 ± 805.6	.105

EAT, epicardial adipose tissue; AS, aortic stenosis.

detected in human calcified valves and accelerates the calcification of human AV interstitial cells obtained from patients with calcific AS [10]. Overall these findings, indicating a potent pro-inflammatory activation of EAT in patients with isolated AS, together with the strong association observed between EAT thickness and AS, support the hypothesis of an involvement of cardiac visceral fat in inflammatory and atherogenic phenomena occurring in the AV and promoting its degeneration and calcification.

Conflicts of interest

None.

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