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Research Article

Extracellular Matrix Remodeling and Inflammation in Transthyretin Amyloidosis

Alessia Argirò[1], Giulia Guidotti[2], Martina Berteotti[2*], Viola Liberati[2], Mattia Zampieri[1], Federico Perfetto[1], Marco Allinovi[1], Anna Poggesi[2], Carlo Di Mario[2], Rossella Marcucci[2], Francesco Cappelli[1]

[1]Department of Clinical and Experimental Medicine, Careggi University Hospital, Florence, Italy; [2]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy

ABSTRACT

Introduction and objectives: Transthyretin Amyloid Cardiomyopathy (ATTR-CM) is an interstitial disease characterized by extracellular deposition of amyloid fibrils. The purpose of the study was to assess if inflammation and Extracellular Matrix (ECM) markers are altered in ATTR, as already known for light chain amyloidosis.

Methods: Fourty-nine patients with ATTR-CM were enrolled in an observational, prospective, cross-sectional, single-center study and underwent clinical, instrumental and biohumoral markers evaluation. The laboratory results were compared to those of an age and sex-matched cohort of 50 patients with sole atrial fibrillation.

Results: Mean age was 78 ± 5 years and 83% of patients were men. Forty-two (86%) had acquired ATTR, 7 (14%) had hereditary ATTR. Patients with ATTR-CM presented higher concentration of inflammatory biomarkers, such as IL-6 (2.9 pg/ml (IQR 1.6-1.33) *vs.* 1.56 pg/ml (IQR 0.43-3.12), p=0.002), IL-8 (17.21 pg/ml (IQR 11.94-34.8) *vs.* 8.54 pg/ml (IQR 5.15-13.3), p<0.001) and VEGF (101.03 pg/ml (IQR 59.05-146.2) *vs.* 62.31 pg/ml (IQR 35.28-112.43), p=0.011), while anti-inflammatory IL-10 was lower (0.2 pg/ml (IQR 0.1-0.2) *vs.* 2.89 pg/ml (IQR 0.33-3.46), p<0.001). Serum levels of metalloprotease MMP-12, TIMP-1 and TIMP-3 were significantly higher in ATTR-CM, with an increased ratio between MMP-12 and TIMP-2, -3 and -4 in ATTR-CM expressing an imbalance towards ECM degradation.

Conclusion: These data suggest that ATTR-CM is characterized by increased inflammation and an unbalance in ECM homeostasis, with degradation prevailing over synthesis of ECM.

Keywords: Transthyretin amyloid cardiomyopathy; Inflammation; Matrix metalloproteinases

Abbreviations: ATTR-CM: Transthyretin Amyloid Cardiomyopathy; AF: Atrial Fibrillation; ECM: Extracellular Matrix; IL: Interleukin; MMP: Metalloproteinases; TIMPS: Tissue Inhibitor of Metallo-Proteinases

INTRODUCTION

Cardiac amyloidosis is a rare disease characterized by extracellular deposition of amyloid fibrils in cardiac tissues. The most common amyloid precursors include monoclonal light chains and their fragments in the context of a plasmacellular dyscrasia in AL amyloidosis, and mutated or senescent transthyretin respectively in hereditary (ATTRv) or acquired (ATTRwt) transthyretin amyloidosis.

The pathophysiological modifications begin in the Extracellular Matrix (ECM), making cardiac amyloidosis an emblem of interstitial disease. The deposition of amyloid substance in the myocardial ECM leads to several detrimental consequences. The accumulation of amyloid fibrils increases myocardial thickness and stiffness, accounting for early diastolic dysfunction. In later stages amyloid deposition may affect electromechanical transduction and myocardial perfusion eventually leading to systolic dysfunction [1].

ECM represents not only a structural support for tissues, but also promotes intercellular communications by producing molecular transducers and growth factors fundamental for cell survival. ECM homeostasis depends on the equilibrium between

Correspondence to: Martina Berteotti, Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy, E-mail: martina.berteotti@unifi.it

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protein degradation and synthesis, respectively mediated by Matrix Metalloproteinases (MMPs) and their inhibitors (TIMPs). In cardiac tissue, an imbalance towards ECM degradation may favour the development of systolic dysfunction, on the other hand, accentuated deposition of ECM may enhance myocardial stiffness and diastolic dysfunction [2]. ECM remodelling and amyloid deposition may concur to trigger an inflammatory response, described in AL and ATTRv related to Val30Met mutation [3,4].

In this study we aimed to characterize the ECM mediators and inflammatory profile of patients with ATTRwt and ATTRv compared to a control population of patients with sole Atrial Fibrillation (AF) matched for age and sex.

MATERIALS AND METHODS

This is an observational, prospective, cross-sectional, single-center study that enrolled 49 patients with Transthyretin Amyloid Cardiomyopathy (ATTR-CM) evaluated at a regional amyloidosis centre. The diagnosis was made according to the latest European guidelines [5]. A diagnosis of ATTR-CM was obtained in presence of typical echocardiography of cardiac magnetic resonance imaging findings, when 99 m Tc-hydroxymethylene diphosphonate scintigraphy showed grade 2 or 3 myocardial uptake of radiotracer and clonal dyscrasia was excluded [5]. All patients underwent clinical evaluation, Electrocardiography (ECG), echocardiography and laboratory evaluation. The control group consisted of 50 patients matched for age and sex enrolled in the strat-AF study. The latter was funded by the Italy Ministry of Health and conducted on patients with AF (Project identification code 16RFAP, approved on March 2017). Our institutional review board, Ethic Committee for clinical experimentation of Tuscany Region, authorized use of this data according to the principles outlined in the Declaration of Helsinki.

Echocardiography

Echocardiography was performed with a Vivid 9 System (Vingmed, General Electric, Horten, Norway) equipped with a 3S probe. Left Ventricle (LV) dimensions and ejection fraction were measured following the current guidelines [6]. Transmitral flow velocity in early (E) and late (A) diastole was measured by pulsed Doppler from the apical four-chamber view [7].

Laboratory test

Blood samples were collected in evacuated plastic tubes Vacutainer and centrifuged at 4°C (2000 x g for 15 min). After centrifugation, the serum was aliquoted, placed in Eppendorf cones and then rapidly frozen in liquid nitrogen and stored at -80°C until the day of analysis. The Bio-Plex Suspension Array System was used for the evaluation of parameters of inflammation and collagenopathy/fibrosis.

Circulating biomarkers of inflammation (IL-1Ra, IL-2, IL-6, IL-8, IL-10, TNF-α, CCL-3, ICAM-1, VCAM-1, VEGF) and extracellular matrix remodelling (MMP-2, MMP-8, MMP-9, MMP-12, TIMP-1, TIMP-2, TIMP-3, TIMP-4, A2-macroglobulin) were measured.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Macintosh, version 27.0 (IBM Corp, USA). Normally distributed continuous data were expressed as mean values ± Standard Deviation (SD). Non-normally distributed continuous data were expressed as median (inter-quartile range, IQR) and categorical variables as frequency (percentage). The unpaired t test or Wilcoxon rank-sum test were used for comparing two groups as appropriate, while chi-squared test was performed for categorical variables. Statistical significance was defined as an $\alpha<0.05$.

RESULTS

Patients with ATTR-CM averaged 78.9 ± 5.6 years and 41 (83.7%) were men (Table 1). Forty-two patients (85.7%) had ATTRwt and 7 (14.3%) had ATTRv (5-Ile68leu, 2-Val122Il mutations).

The control population consisted of 50 patients with AF, with a mean age of 79.15 ± 5.4 years and 40 (80%) were men. Patients in the control group showed a greater CHA2DS2-VASc score (3.0 ± 0.64 *vs.* 3.6 ± 1, p<0.001) and had more frequently a smoking history (8% *vs.* 58%, p<0.001). In Table 2 ECG and echocardiography characteristics of patients with ATTR-CM and controls are reported. Pacemaker implantation was more frequent in ATTR-CM compared to controls (20% *vs.* 0%, p<0.012). Patients with ATTR-CM had smaller LV cavity (end diastolic volume 86 ml (IQR 72-105) *vs.* 104 ml (IQR 52-170), p=0.016), lower ejection fraction (55% (IQR 47-60) *vs.* 59% (IQR 37-68) p=0.001), and worse diastolic parameters compared to controls.

Table 3 shows the comparison of biohumoral markers between ATTR-CM patients and controls. Inflammatory interleukins were significantly higher in patients with ATTR-CM compared to controls: IL6 (2.9 pg/ml (IQR 1.6-1.33) *vs.* 1.56 (IQR 0.43-3.12), p<0.01], IL8 (17.21 pg/ml (IQR 11.94-34.8) *vs.* 8.54 (IQR 5.15-13.3), p<0.01) and VEGF (101.03 pg/ml (IQR 59.05-146.2) *vs.* 62.31 pg/ml (35.28-112.43), p<0.01). On the other side, the concentration of the anti-inflammatory IL-10 was decreased in patients with ATTR-CM compared to controls (0.2 pg/ml (IQR 0.1-0.2) *vs.* 2.89 pg/ml (IQR 0.3-3.46), p<0.001). MMP-12 was significantly increased in ATTR-CM compared to controls [1060 ng/ml (IQR 745-1260) *vs.* 414 ng/ml (50-612), p<0.001). The MMP inhibitors were increased in ATTR compared to controls: TIMP-1 (177 ng/ml (158-212) *vs.* 157 ng/ml (IQR 130-201), p=0.03) and TIMP-3 (69 ng/ml (IQR 47.88-92.79) *vs.* 36.11 ng/ml (IQR 28.71-55.9), p<0.01). In Table 4 the ratios between MMPs and their inhibitors are reported. Notably, the ratio between MMP-12 and its inhibitors, TIMP-2, TIMP-3 and -4, was higher is ATTR-CM compared to controls expressing an enhanced ECM degradation over synthesis (Figure 1).

Table 1: Baseline characteristics of patients and controls.

	Patients (n=49)	Controls (n=50)	P value
Age, mean ± SD	78.9 ± 5.6	79.15 ± 5.4	0.809
Male, n (%)	41 (83.7%)	40 (80%)	0.636
ATTRwt, n (%)	42 (85.7%)	-	-
ATTRv, n (%)	7 (14.3%)	-	-
Ile68Leu, n (%)	5 (10.2%)	-	-
Val122Ile, n (%)	2 (4.1%)	-	-
Hypertension, n (%)	28 (57.14%)	39 (78%)	0.027
Diabetes, n (%)	7 (14.29%)	3 (6%)	0.171
Dyslipidemia, n (%)	26 (53.06%)	20 (40%)	0.193

Previous smoke, n (%)	4 (8.16%)	29 (58%)	<0.001
BMI, mean ± SD	25.3 ± 3.2	25.8 ± 4	0.503
CHA2SD2-VASc, mean ± SD	3 ± 0.64	3.6 ± 1.7	<0.001
PAD, n (%)	2 (4.1%)	4 (8%)	0.414
CAD, n (%)	8 (16.4%)	11 (22%)	0.111
TIA/Ischemic stroke, n (%)	8 (16.3%)	8 (16%)	0.965
Hemorrhagic stroke, n (%)	0 (0%)	2 (4%)	0.157
NYHA class, n (%)	49 (100%)	7 (14%)	<0.001
I, n (%)	17 (34.7%)	0(0%=	<0.001
II, n (%)	21 (42.86%)	6(0.12%)	<0.001
III, n (%)	11 (22.45%)	1 (0.02%)	0.002
Creatinine (mg/dl), mean ± SD	1.57 ± 1.99	0.96 ± 0.24	0.167
NT-proBNP (pg/mL), mean ± SD	3209.82 ± 3205.12	-	-
Furosemide, n (%)	48 (98%)	14 (28%)	<0.001
Furosemide daily dose	-	-	-
<100 mg/die, n (%) 100-200 mg/die, n (%) >200 mg/die, n (%)	39 (80%) 6 (12%) 3 (6%)		
Ace-I/ARB, n (%)	9 (18%)	19 (38%)	0.03
MRA, n (%)	12 (24%)	0 (0%)	<0.001
Calcium antagonists, n (%)	11 (22%)	12 (24%)	0.855
Beta-blockers, n (%)	22 (45%)	13 (26%)	0.049
Amiodarone, n (%)	4 (8%)	2 (4%)	<0.001
Digoxin, n (%)	1 (2%)	8 (16%)	<0.001
Coumadin, n (%)	4 (8%)	14 (28%)	0.011
NOAC, n (%)	27 (55%)	36 (72%)	0.081
Antiplatelet, n (%)	7 (14%)	26 (52 %)	<0.001
SGLT2 inhibitors, n (%)	7 (14%)	0 (0%)	0.003
Statin +/- ezetimibe, n (%)	23 (47%)	12 (24%)	0.002
Ezetimibe, n (%)	2 (4%)	3 (6%)	0.663
Evolocumab, n (%)	1 (2%)	0 (0%)	0.31
Tafamidis, n (%)	12 (25%)	-	-

Note: ATTRwt: Wild type Transthyretin Amyloidosis; ATTRv: variant Transthyretin Amyloidosis; BMI: Body Mass Index; PAD: Peripheral Artery Disease; CAD: Coronary Artery Disease; TIA: Transient Ischemic Attack; NYHA: New York Heart Association; NT-proBNP: N-Terminal pro-Brain Natriuretic Peptide; ACE-I/ARB Angiotensin Converting Enzyme Inhibitors/Angiotensin Receptor Blocker; MRA: Mineralocorticoid Receptor Antagonist; NOAC: Non vitamin K Oral Anticoagulants; SGLT2i: Sodium-Glucose Cotransporter-2 Inhibitors.

Table 2: Electrocardiography and echocardiography characteristics of patients and controls.

	Patients (n=49)	Controls (n=27)	P value
Electrocardiography			
Atrial fibrillation, n (%)	31 (61%)	27 (100%)	<0.001
Pacemaker, n (%)	10 (20%)	0 (0%)	0.012
First degree AV block, n (%)	9 (18%)	4 (8%)	0.694
LBBB, n (%)	6 (12%)	1 (2%)	0.218
RBBB, n (%)	7 (14%)	1 (2%)	0.15
Heart rate (bpm), median (IQR)	70 (64-76)	70 (48-90)	0.468
Echocardiography			
LVESV (ml), median (IQR)	40 (31-50)	43 (18-74)	0.762
LVEDV (ml), median (IQR)	86 (72-105)	104 (52-170)	0.016
EF (%), median (IQR)	55 (47-60)	59 (37-68)	0.001
LA Volume (ml), median (IQR)	90 (70-108)	99 (43-212)	0.288
E (cm/sec), median (IQR)	77 (66-91)	82 (47-124)	0.741
A (cm/sec), median (IQR)	55 (43-85)	76 (28-122)	0.164
DT (msec), median (IQR)	178 (135-216)	223 (60-344)	0.003
Septal e' (cm/sec), median (IQR)	5 (5-7)	9 (4-14)	<0.001
Lateral e' (cm/sec), median (IQR)	4 (4-6)	11 (6-13)	<0.001

Note: AV: Atrioventricular, LBBB: Left Bundle Branch Block, RBBB: Right Bundle Branch Block, LV: Left Ventricular, ESV: End-Systolic Volume, EDV: End-Diastolic Volume, EF: Ejection Fraction, DT: Deceleration Time.

Table 3: Extracellular matrix and inflammatory biomarkers in patients and controls.

Biomarkers	Patients (n=49)	Controls (n=50)	p
MMP-2 (ng/ml)	577.04 (448.64-681.33)	543.94 (456.14-652.4)	0.969
MMP-8 (ng/ml)	9.52 (5.58-14.86)	8.02 (4.82-15.97)	0.529
MMP-9 (ng/ml)	330.1 (220.62-428.76)	324.64 (207.55-558.71)	0.424
MMP-12 (ng/ml)	1060.0 (745.0-1260.0)	414.58 (49.73-612.28)	<0.001
TIMP-1 (ng/ml)	177.26 (158.08-212.36)	157.94 (129.84-201.01)	0.038
TIMP-2 (ng/ml)	139.47 (122.01-171.01)	133.22 (95.31-198.06)	0.329
TIMP-3 (ng/ml)	68.78 (47.88-92.79)	36.11 (28.71-55.9)	<0.001
TIMP-4 (ng/ml)	3.64 (2.6-5.31)	3.5 (2.23-5.87)	0.539
IL-6 (pg/ml)	2.9 (1.61-1.33)	1.56 (0.43-3.12)	0.002

IL-8 (pg/ml)	17.21 (11.94-34.08)	8.54 (5.15-13.03)	<0.001
IL-10 (pg/ml)	0.2 (0.1-0.2)	2.89 (0.33-3.46)	<0.001
TNF-α (pg/ml)	2.8 (1.43-5.42)	2.21 (0.98-4.01)	0.134
ICAM-1 (ng/ml)	266.51 (217.55-397.94)	313.23 (268.74-406.85)	0.101
VCAM-1 (ng/ml)	1422.1 (951.1-1991.4)	1514.7 (1047.2-2128.48)	0.547
VEGF (pg/ml)	101.09 (59.05-146.42)	62.31 (35.28-112.43)	0.011

Note: Data are expressed as median (IQR). MMP: Matrix Metalloproteinases, TIMP: Tissue Inhibitors of Metalloproteinases, IL: Interleukin, TNF: Tumor Necrosis Factor, ICAM: Intercellular Adhesion Molecule, VCAM: Vascular Cell Adhesion Molecule, VEGF: Vascular Endothelial Growth Factor.

Table 4: Rations between matrix metalloproteinases and tissue inhibitors of metalloproteinases.

Biomarkers	Patients (n=49)	Controls (n=50)	P value
MMP2/TIMP1	2.98 (2.22-3.81)	3.49 (2.63-4.44)	0.103
MMP2/TIMP2	3.81 (3.36-4.19)	4.24 (3.09-5.44)	0.059
MMP2/TIMP3	8.29 (5.77-11.13)	15.27 (10.29-22.66)	<0.001
MMP2/TIMP4	143.53 (112.87-203.42)	178.9 (117.33-209.76)	0.24
MMP8/TIMP4	2.76 (1.55-4.52)	2.66 (1.29-6.09)	0.89
MMP8/TIMP3	0.15 (0.07-0.23)	0.24 (0.16-0.38)	0.002
MMP8/TIMP2	0.06 (0.04-0.1)	0.07 (0.04-0.11)	0.98
MMP8/TIMP1	0.05 (0.04-0.08)	0.05 (0.03-0.08)	0.884
MMP9/TIMP1	1.66 (1.15-2.31)	2.02 (1.36-2.88)	0.063
MMP9/TIMP2	2.14 (1.42-2.89)	2.28 (1.58-3.71)	0.234
MMP9/TIMP3	5.07 (2.35-7.54)	9.74 (6.73-12.7)	<0.001
MMP9/TIMP4	77.44 (54.38-135.53)	91.83 (61.53-181.73)	0.197
MMP12/TIMP1	0.0056 (0.0039-0.0071)	0.0022 (0.0004-0.0032)	<0.001
MMP12/TIMP2	0.0068 (0.0050-0.0088)	0.0023 (0.0005-0.0040)	<0.001
MMP12/TIMP3	0.0166 (0.0096-0.224)	0.0081 (0.0015-0.0158)	<0.001
MMP12/TIMP4	0.2649 (0.1926-0.4316)	0.0795 (0.0220-0.1374)	<0.001

Note: Data are expressed as median (IQR). MMP: Matrix Metalloproteinases, TIMP: Tissue Inhibitors of Metalloproteinases.

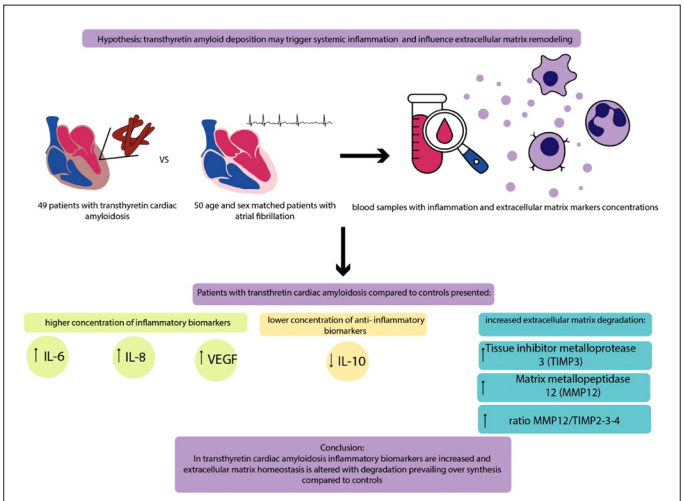

Figure 1: Fourty-nine patients with ATTR-CM were compared with matched control with atrial fibrillation, showing that ATTR-CM is characterized by increased inflammation and an unbalance in ECM homeostasis, with degradation prevailing over synthesis of ECM.

DISCUSSION

In this study we compared inflammatory and ECM biomarkers in patients with ATTR-CM and sole AF. The main results of the study include: (1) patients with ATTR-CM present higher concentration of inflammatory biomarkers compared to controls (IL-6, IL-8 and VEGF), (2) In ATTR-CM the anti-inflammatory interleukin IL-10 is lower compared to what measured in patients with sole AF, (3) Serum levels of MMP-12, TIMP-1 and TIMP-3 are higher in ATTR-CM compared to controls, (4) ECM MMP prevail over MMP inhibitors favouring ECM degradation.

Although cardiac amyloidosis is an exemplar interstitial disease in whom the first pathophysiological alteration is the deposition of amyloid fibrils in the ECM, information regarding the role of ECM homeostasis in this setting is rather scarce and focalized more on the comparison between AL and ATTR [8].

In a cohort including 40 patients with ATTR and 10 patients with AL, MMP-9 and TIMP-1 levels were more elevated in AL compared to ATTR and associated with worse diastolic function [9]. Fifty patients with AL and 50 with ATTR have been evaluated in a further study in which MMP-2 and TIMP-1 levels were higher in AL compared to ATTR, while MMP-9 and TIMP-2 levels were similar between the two subtypes of cardiac amyloidosis.

Furthermore, the ratio of MMP-2/TIMP-2 was higher in AL compared to ATTR, whereas the ratio of MMP-9/TIMP-1 was similar between the groups [10]. These results suggest an increased ECM degradation and disruption in AL compared to ATTR, consistent with the greater level of inflammation due to light chains related cytotoxicity [11].

In the present study MMP inhibitors such as TIMP-1 and TIMP-3 were higher in ATTR-CM compared to controls. These molecules, in particular TIMP-1, may favour cardiac fibrosis through the CD63/β1 cascade that activates profibrotic signalling in cardiac fibroblasts [12]. In fact, fibroblasts cultured on TTR-deposited substrates show cytoskeletal and nuclear architecture dysregulation, fewer focal adhesion and increased proliferation that eventually result in increased fibrosis [13].

The prevalent fibrotic component in cardiac amyloidosis has been underlined in a recent study focusing on endomyocardial

biopsies: Myocardial fibrosis occupied the same amount of tissue as amyloid deposits (around 15%) with similar distribution between ATTR and AL [14]. Thus, the presence of both amyloid and fibrosis may justify the increase in extracellular volume reported by cardiac magnetic resonance and influence prognosis [14].

Furthermore, it has already been demonstrated that fibroblasts exposed to TTR express higher levels of inflammatory cytokines (CXCL-1, CXCL-2, CXCL-3, CXCL-5, CCL-3, CCL-5, CCL-20) and MMP such as MMP-12, which was found elevated also in our study [13] MMP-12 is produced mostly by macrophages and degrades elastin, fibronectin, laminin and type IV collagen [15]. Moreover, MMP-12 activates the proinflammatory TNF-α [15] and its levels have been associated with cardiovascular disease, atherosclerotic burden and plaque inflammation [16].

In our study we noticed a predominance of ECM degradation over formation that might be interpreted as an attempt to remove amyloid deposits. In fact, MMP-9 is capable of degrading TTR aggregated and fibrils *in vitro*, but, for yet unknown reasons, the same mechanism is not effective *in vivo* [17].

In our study we confirmed the presence of a proinflammatory environment in patients with ATTR-CM characterized by higher levels of the proinflammatory cytokines IL-6 and IL-8 and reduced concentration of the anti-inflammatory IL-10 compared to controls [18]. Other studies conducted in familial amyloid polyneuropathy patients demonstrated that TTR deposition induces proinflammatory cascade, [17] which promotes disease progression [3]. In a recent study of 28 patients with ATTRv related to Val30Met mutation, the levels of inflammatory (TNF-α, IL-1β, IL-8, IL-33, IFN-β) and anti-inflammatory (IL-10) cytokines were increased compared to healthy age-matched controls. Interestingly, even asymptomatic patients showed high levels of IL-33, IL-1β and IL-10 suggesting that inflammation may be an early disease manifestation [3].

We also observed significantly higher values of VEGF, which is an angiogenic cytokine necessary for vascular development and repair. VEGF has been compared among patients with AL-CM (n=26), ATTR-CM (n=7), non-cardiac systemic amyloidosis (n=7), left ventricular hypertrophy (n=45) and systolic heart failure (n=42), showing a trend towards higher levels in ATTR-CM (p=0.057). In our study the significance was probably reached due to a higher number of enrolled subjects and suggests that VEGF may be related to the inflammatory response and/or the microvascular dysfunction.

Hence, we can appreciate that ECM and inflammation are profoundly altered in ATTR-CM, creating a complex environment in which probably amyloid deposition triggers inflammation and ECM remodeling, that in turn enhance each other generating a vicious circle.

This study has several limitations, first, the limited sample size and the single centre source of patients may limit the generalization of our finding. Second, the control cohort did not include healthy subjects. However, a higher burden of inflammation has extensively been described in AF patients, therefore reinforcing the findings of the present study with AF [19]. Finally, the studied population was mainly composed by men, affecting generalizability. However, in patients with ATTRwt cardiac amyloidosis a male to female ratio of 7:1 has already been described, [20] confirming that the enrolled population probably depicts a real-world population affected by this condition.

CONCLUSION

In patients with ATTR-CM inflammatory cytokines such as IL-6, IL-8 and VEGF were increased while the anti-inflammatory IL-10 was reduced compared to controls generating a proinflammatory environment. The ECM remodeling biomarkers were increased with ECM degradation prevailing over ECM synthesis. Therefore, we recognize that ATTR-CM results in significant changes to both ECM and inflammation. These changes create a complicated environment where amyloid deposition likely initiates inflammation and ECM remodeling, which then feed off each other to create a vicious cycle.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest related to this work.

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REFERENCES

1. Dorbala S, Vangala D, Bruyere J, Quarta C, Kruger J, Padera R, et al. Coronary microvascular dysfunction is related to abnormalities in myocardial structure and function in cardiac amyloidosis. JACC Heart Fail. 2014;2(4):358-367.
2. Frangogiannis NG. The extracellular matrix in ischemic and nonischemic heart failure. Circ Res. 2019;125(1):117-146.
3. Azevedo EP, Guimaraes-Costa AB, Bandeira-Melo C, Chimelli L, Waddington-Cruz M, Saraiva EM, et al. Inflammatory profiling of patients with familial amyloid polyneuropathy. BMC Neurol. 2019;19;1-7.
4. Siegismund CS, Escher F, Lassner D, Kühl U, Gross U, Fruhwald F, et al. Intramyocardial inflammation predicts adverse outcome in patients with cardiac AL amyloidosis. Eur J Heart Fail. 2018;20(4):751-757.
5. Garcia-Pavia P, Rapezzi C, Adler Y, Arad M, Basso C, Brucato A, et al. Diagnosis and treatment of cardiac amyloidosis. A position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur J Heart Fail. 2021;23(4):512-526.
6. Lang RM, Badano LP, Victor MA, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015;28(1):1-39.
7. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2016;29(4):277-314.
8. Fontana M, Ćorović A, Scully P, Moon JC. Myocardial amyloidosis: The exemplar interstitial disease. JACC Cardiovasc Imaging. 2019;12:2345-2356.

9. Biolo A, Ramamurthy S, Connors LH, O'Hara CJ, Meier-Ewert HK, Soo Hoo PT, et al. Matrix metalloproteinases and their tissue inhibitors in cardiac amyloidosis: Relationship to structural, functional myocardial changes and to light chain amyloid deposition. Circ Heart Fail. 2008;1(4):249-257.

10. Tanaka K, Essick EE, Doros G, Tanriverdi K, Connors LH, Seldin DC, et al. Circulating matrix metalloproteinases and tissue inhibitors of metalloproteinases in cardiac amyloidosis. J Am Heart Assoc. 2013;2(2):5868.

11. Kotecha T, Martinez-Naharro A, Treibel TA, Francis R, Nordin S, Abdel-Gadir A, et al. Myocardial edema and prognosis in amyloidosis. J Am Coll Cardiol. 2018;71:2919-2931.

12. Takawale A, Zhang P, Patel VB, Wang X, Oudit G, Kassiri Z. Tissue inhibitor of matrix metalloproteinase-1 promotes myocardial fibrosis by mediating CD63-integrin β1 interaction. Hypertension. 2017;69:1092-1103.

13. Dittloff KT, Iezzi A, Zhong JX, Mohindra P, Desai TA, Russell B. Transthyretin amyloid fibrils alter primary fibroblast structure, function, and inflammatory gene expression. Am J Physiol Heart Circ Physiol. 2021;321(1):149-160.

14. Pucci A, Aimo A, Musetti V, Barison A, Vergaro G, Genovesi D, et al. Amyloid deposits and fibrosis on left ventricular endomyocardial biopsy correlate with extracellular volume in cardiac amyloidosis. J Am Heart Assoc. 2021;10(20):20358.

15. Chandler S, Cossins J, Lury J, Wells G. Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-α fusion protein. Biochem Biophys Res Commun. 1996;228(2):421-429.

16. Goncalves I, Bengtsson E, Colhoun HM, Shore AC, Palombo C, Natali A, et al. Elevated plasma levels of MMP-12 are associated with atherosclerotic burden and symptomatic cardiovascular disease in subjects with type 2 diabetes. Arterioscler Thromb Vasc Biol. 2015;35(7):1723-1731.

17. Sousa MM, Amaral JB, Guimarães A, Saraiva MJ. Up-regulation of the extracellular matrix remodeling genes, biglycan, neutrophil gelatinase-associated lipocalin and matrix metalloproteinase-9 in familial amyloid polyneuropathy. FASEB J. 2005;19:124-126.

18. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol. 2011;29:71-109.

19. Guo Y, Lip GYH, Apostolakis S. Inflammation in atrial fibrillation. J Am Coll Cardiol. 2012;60(22):2263-2270.

20. Kroi F, Fischer N, Gezin A, Hashim M, Rozenbaum MH. Estimating the gender distribution of patients with wild-type transthyretin amyloid cardiomyopathy: A systematic review and meta-analysis. Cardiol Ther. 2021;10(1):41-55.