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TESE DE DOUTORADO

ESTUDO DE FATORES ENVOLVIDOS NO REMODELAMENTO VENTRICULAR EM PACIENTES COM DIFERENTES FORMAS CLÍNICAS DE INSUFICIÊNCIA CARDÍACA

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Introdução

A insuficiência cardíaca (IC) é um importante problema de saúde em todo o mundo, e apesar dos muitos avanços terapêuticos recentes, permanece associada a elevada morbidade e mortalidade ([1;2]). Nos Estados Unidos da América, a IC resultou em mais de um milhão de hospitalizações em 2007, sendo que os gastos com seu diagnóstico e tratamento suplantam aqueles relacionados a qualquer outra doença ([3]). No Brasil, a IC resulta em cerca de 300000 internações por ano sendo a principal causa de internação em pacientes com mais de 60 anos, com uma mortalidade hospitalar em torno de 8% ([4]).

1. Insuficiência cardíaca e remodelamento miocárdico

Insuficiência cardíaca (IC) é definida como uma condição em que o coração é incapaz de manter débito cardíaco suficiente para suprir as necessidades metabólicas tissulares ou o faz às custas de pressões de enchimento elevadas. Clinicamente, a IC é uma síndrome com manifestações que resultam tanto de um estado de baixo débito cardíaco e congestão como de mecanismos compensatórios mal-adaptativos ([2]).

Nas últimas décadas, o conceito de IC, antes primordialmente clínico, evoluiu para uma definição que incorpora os mecanismos envolvidos em seu desenvolvimento e progressão. Assim, a IC é vista como uma doença progressiva, iniciada após um insulto cardíaco com conseqüente redução na função miocárdica e ativação de sistemas compensatórios que, agudamente,

são capazes de restabelecer a função cardíaca a níveis próximos dos normais. Entretanto, a ativação sustentada destes sistemas resulta em dano miocárdico adicional com piora progressiva da função miocárdica. Ocorrem alterações no tamanho, forma e função miocárdicos, conhecidos como remodelamento cardíaco, fundamental para o desenvolvimento e progressão da IC. Os processos envolvidos no remodelamento cardíaco são complexos e incluem alterações tanto no cardiomiócito como na matriz extracelular ([5;6]).

1.1 Cardiomiócito e morte celular

Alterações nos cardiomiócitos, especialmente hipertrofia e morte celular por necrose ou apoptose, são vistas como centrais no processo de remodelamento miocárdico ([6]). A morte celular tem sido implicada na progressão do remodelamento, e a apoptose parece ser parte de um mecanismo regulatório envolvido na resposta adaptativa a insultos cardíacos ([7]).

O uso de troponinas na avaliação de lesão e morte de cardiomiócitos está bem estabelecido, sendo estes os biomarcadores de escolha para a detecção de necrose miocárdica na suspeita de infarto do miocárdio ([8]). As troponinas são proteínas que regulam interações entre actina e miosina durante a contração muscular. As troponinas T e I têm isoformas distintas nos músculos esquelético e cardíaco, e desta forma é possível a detecção de isoformas cardíacas específicas. O reconhecimento da presença de lesão e morte de cardiomiócitos, por meio de marcadores específicos como as troponinas cardíacas, traz a

possibilidade de detecção e monitoramento deste processo e sua contribuição para o remodelamento e progressão da IC.

O aumento nos níveis de troponina foi demonstrado em pacientes com IC grave ([9;10]) e parece estar associado a doença avançada e a pior prognóstico ([10-14]). Em pacientes com IC descompensada, diversos estudos demonstram que níveis elevados de troponina se associam a maior mortalidade ([13;15-18]). Entretanto, a proporção de pacientes com troponina detectável varia de forma importante entre os diferentes estudos, dependendo, entre outros fatores, das características dos pacientes selecionados e das diferentes técnicas ou sensibilidade dos ensaios utilizados, e se desconhece se episódios de descompensação resultam em lesão adicional de cardiomiócitos em comparação àquela presente em pacientes com IC compensada. Desta forma, o uso deste biomarcador poderia trazer informações sobre lesão e morte de cardiomiócitos em diferentes cenários da doença, auxiliando no entendimento da fisiopatologia envolvida na progressão da IC ([19]).

1.2 Matriz extracelular

A matriz extracelular miocárdica é uma estrutura complexa contendo várias proteínas, moléculas sinalizadoras, proteases e células. Os principais componentes da matriz são os colágenos fibrilares tipos I e III, cujos conteúdo, geometria e quantidade relativa são determinantes da integridade estrutural e funcional do músculo cardíaco ([20;21]). Além de fornecer suporte estrutural e funcional para o miocárdio, a matriz extracelular é também um microambiente

dinâmico para sinalização celular ([22]). Sua composição é determinada pelo equilíbrio entre o constante processo de síntese e degradação dos elementos que a compõem. A degradação de colágeno é por sua vez regulada pela atividade das metaloproteinases da matriz (MMPs), uma família de enzimas intersticiais zinco-dependentes, e de seus inibidores teciduais (TIMPs). Tanto o processo de síntese como a degradação de colágeno são regulados por neurohormônios ([23;24]), citocinas inflamatórias ([25-27]), estresse oxidativo ([28;29]) e estiramento mecânico ([30]), fatores presentes no miocárdio, particularmente durante a progressão da IC.

Mudanças na expressão e/ou atividade das MMPs e TIMPs contribuem para a patogênese da IC, estando presentes em pacientes com IC de etiologias diversas ([31-35]). Da mesma forma, níveis alterados de peptídeos N-terminais liberados no processo de síntese de colágeno têm sido demonstrados em pacientes com IC ([36-38]). Além disso, estes marcadores de ativação da matriz extracelular se associam a remodelamento cardíaco e a desfechos desfavoráveis na IC crônica ([36-38]).

A importância da matriz extracelular no desenvolvimento e progressão da IC foi também demonstrada em modelos experimentais de doença cardíaca ([22]). O uso de modelos experimentais de IC, de animais transgênicos e de inibidores endógenos ou farmacológicos de MMPs traz evidências de uma relação causa/efeito entre a atividade da matriz extracelular e o remodelamento cardíaco. Enquanto a expressão miocárdica aumentada de MMP resulta em dilatação e disfunção ventricular, inibidores de MMPs e nocaute gênico afetam

favoravelmente o remodelamento cardíaco, sugerindo que esta possa ser uma terapia potencial para pacientes com IC ou em risco para seu desenvolvimento ([22;39-42]).

Entretanto, existem resultados heterogêneos sobre o papel da matriz extracelular na IC, e o primeiro ensaio clínico realizado para avaliar o efeito da inibição de MMPs em pacientes com IC não trouxe resultados positivos ([43]). O melhor entendimento de quais fatores, condições ou etiologias resultam em maior ou menor ativação deste sistema poderia nortear estudos futuros e intervenções potencialmente efetivas para a prevenção manejo da IC.

1.3 Rigidez arterial e o acoplamento ventricular-arterial

O sistema arterial desempenha duas funções principais: a) como um tampão elástico, se expande e recebe o fluxo pulsátil de alta pressão na aorta proximal e o transforma em um fluxo contínuo e de baixa pressão quando a onda de pulso atinge o leito capilar; b) como um condutor, facilita a propagação do fluxo sanguíneo para a periferia durante a diástole, quando o sistema arterial “recua” para seu estado basal. O aumento da carga imposta pelo sistema arterial está implicado na fisiopatologia de muitas doenças cardiovasculares, incluindo a IC ([44]). O aumento da rigidez arterial traz vários efeitos deletérios sobre a função ventricular: a pós-carga imposta ao coração é maior, a eficiência da ejeção ventricular é reduzida e a perfusão do próprio miocárdio é também reduzida ([45;46]). Na IC, o coração é especialmente sensível a alterações no sistema

arterial, e desta forma o acoplamento ventricular-arterial passa a ser um importante determinante da função cardíaca.

A avaliação da rigidez arterial é limitada pela ausência de uma definição quanto ao melhor método para sua determinação ([47]). Uma vez que o enrijecimento do sistema arterial resulta em pressão de pulso elevada, esta tem sido utilizada como um indicador de rigidez arterial. Pressão de pulso elevada está associada ao desenvolvimento de doença cardiovascular e IC ([48;49]), e a pior prognóstico em pacientes com disfunção de ventrículo esquerdo e IC sintomática ([50;51]).

Outra forma de avaliar a rigidez arterial é através da medida da velocidade da onda de pulso (VOP). A contração ventricular gera uma onda de pulso que chega à periferia a uma determinada velocidade; com o enrijecimento da parede arterial, esta onda de pulso é transmitida mais rapidamente, de forma que a VOP será maior. Diversos estudos sugerem aumento na rigidez arterial e alterações no acoplamento ventricular-arterial em pacientes com IC, com maior dependência do ventrículo esquerdo no sistema arterial ([52-54]). Em pacientes com IC crônica estável, este aumento na rigidez arterial parece não ser homogêneo em todo o leito arterial, predominando em leitos centrais, enquanto no leito arterial periférico parece haver até mesmo redução na rigidez arterial ([55]). Todos esses achados sugerem que o aumento na rigidez arterial e o acoplamento ventricular-arterial anormal possa desempenhar papel importante na fisiopatologia da IC. Entretanto, mais estudos são necessários para avaliar a contribuição de diferentes segmentos do sistema arterial e a presença de

alterações dinâmicas destas características em situações clínicas diversas no contexto da IC sistólica.

2. Situações clínicas peculiares

O conhecimento dos processos envolvidos no remodelamento cardíaco mudou o paradigma do tratamento da IC crônica, de uma ênfase inicial na melhora de sintomas e parâmetros hemodinâmicos (diuréticos, inotrópicos, vasodilatadores), para intervenções que retardam a progressão da doença e aumentam sobrevida, primariamente por interromper processos envolvidos no remodelamento e piora progressiva da função miocárdica (beta-bloqueadores, inibidores da enzima conversora de angiotensina, antagonistas da aldosterona). Entretanto, diversas situações clínicas específicas e etiologias menos freqüentes têm sido pouco estudadas, e o melhor entendimento destas situações pode contribuir para o desenvolvimento de estratégias terapêuticas mais adequadas. A seguir, salientamos duas destas condições.

2.1 Insuficiência Cardíaca Agudamente Descompensada (ICAD)

O tratamento da ICAD mudou muito pouco nas últimas décadas, e continua tendo como foco a melhora sintomática e os parâmetros hemodinâmicos, com o uso de diuréticos, vasodilatadores e inotrópicos. Embora a melhora sintomática e hemodinâmica seja um objetivo importante no curto prazo, a incorporação de um novo paradigma no enfoque terapêutico da ICAD

visando melhorar o prognóstico e evitar a progressão da doença é reconhecida como necessária e foco de muita discussão ([56;57]).

Especula-se que a sobrecarga de volume seja apenas um aspecto da ICAD, e que alerações não apenas cardíacas como também vasculares, neurohumorais e inflamatórias possam estar envolvidos na fisiopatologia dos episódiso de descompensação ([58;59]). Episódios de ICAD estão associados a pressões de enchimento elevadas, ativação de sistemas neurohumorais e aumento em citocinas inflamatórias e extresse oxidativo ([60;61]). Estes estímulos têm efeito sobre processos intrinsecamente relacionados ao remodelamento cardíaco, sendo possível que episódios de descompensação sejam de fato acompanhados por aceleração do remodelamento cardíaco e progressão da IC. Da mesma forma, alterações na rigidez arterial e pós-carga durante episódios de descompensação podem influenciar diretamente a eficiência ventricular, contribuindo para sua complexa fisiopatoloiga e adicionando um possível alvo terapêutico para pacientes com ICAD. Entretanto, somente após entendermos as diferenças entre os estados compensado e descompensado, e os mecanismos fisiopatológicos pelos quais episódios ICAD são deletérios para a progressão da IC é que poderemos ter como objetivo direto a interrupção destes processos.

2.2. Amiloidose cardíaca

Amiloidose cardíaca é uma doença rara que consiste na deposição extracelular de fibrilas amilóides no coração ([62]). Esta deposição resulta em uma

cardiomiopatia restritiva que progride para IC e arritmias. Existem vários tipos de amiloidose cardíaca, classificados de acordo com a natureza bioquímica do depósito amilóide. A seguir, são descritas as formas que mais comumente resultam em doença clinicamente significante:

1. Primária: consiste na forma mais comum de amiloidose cardíaca, onde as fibrilas amilóides são derivadas de imunoglobulinas monoclonais de cadeias leves. Apesar de ser uma doença sistêmica, com acometimento de vários órgãos, insuficiência cardíaca e arritmias são a causa de óbito em mais de 50% dos pacientes;
2. Hereditária sistêmica: é causada pela deposição de fibrilas amilóides derivadas de variantes genéticas da transtirretina, uma proteína transportadora sintetizada no fígado e plexo coroidal;
3. Sistêmica senil: esta forma resulta da deposição de fibrilas amilóides derivadas da transtirretina normal.

Embora o comprometimento estrutural miocárdico seja similar nas diversas formas de amiloidose cardíaca, o comportamento da amiloidose primária é muito mais agressivo, com uma sobrevida de 13 meses (4 se IC presente no diagnóstico) comparada a mais de 70 meses para as demais formas de amiloidose cardíaca ([62-64]). Uma hipótese para explicar esta diferença na evolução clínica é a de que as imunoglobulinas de cadeia leve tenham um papel direto na patogênese da amiloidose primária ([65]). Evidências experimentais demonstram que as cadeias leves têm efeitos deletérios diretos sobre o

miocárdio, e que estes efeitos poderiam ocorrer através da geração de espécies reativas do oxigênio ([66]).

Uma hipótese alternativa ou complementar incluiria o papel da matriz extracelular na progressão da amiloidose cardíaca. Uma vez que a infiltração amilóide no interstício resulta em alterações da matriz extracelular com enrijecimento parietal, este processo está intrinsecamente ligado ao remodelamento cardíaco na amiloidose. Por outro lado, as metaloproteinases da matriz (MMPs) são estimuladas e reguladas por espécies reativas do oxigênio, e poderiam desta forma estar ativadas de forma preferencial na presença de imunoglobulinas de cadeias leves (amiloidose primária), contribuindo para sua progressão clínica acelerada. Não existem, até o momento, estudos clínicos que avaliem o papel de marcadores de atividade proteolítica da matriz ou de seus níveis teciduais nas diversas formas de amiloidose cardíaca.

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Racional dos estudos

Nas últimas décadas, houve importantes avanços no conhecimento dos mecanismos envolvidos no remodelamento cardíaco, bem como dos determinantes da função e eficiência ventriculares, conhecendo-se aspectos importantes para a progressão da IC. A ativação inicialmente compensatória mas subseqüentemente deletéria de sistemas neuro-hormonais tornou-se um alvo terapêutico fundamental no manejo de pacientes com IC sistólica. O conhecimento de processos celulares envolvidos no remodelamento cardíaco (como dano aos cardiomiócitos e alterações na matriz extracelular), e da contribuição de outros órgãos como o sistema arterial, traz um novo paradigma para o entendimento desta síndrome e, possivelmente, para o desenvolvimento de estratégias cardioprotetoras.

Entretanto, apesar dos muitos estudos realizados no contexto da IC sistólica crônica, pouco se conhece sobre a fisiopatologia da IC agudamente descompensada e suas diferenças com relação ao estado compensado, e sobre formas menos comuns de IC como a amiloidose cardíaca. Desta forma, o presente estudo procura avaliar alguns destes processos em diferentes contextos clínicos da IC, procurando contribuir para o conhecimento de aspectos fisiopatológicos associados a estas condições. Este é um passo fundamental para que se possa avançar na procura por intervenções mais efetivas para o manejo destas situações.

Objetivos

Objetivo Geral

Avaliar mecanismos envolvidos no processo de remodelamento cardíaco em pacientes com diferentes formas clínicas de insuficiência cardíaca.

Objetivos específicos

I. Avaliar marcadores de lesão de cardiomiócitos e de atividade da matriz extracelular em pacientes com ICAD comparados a pacientes com IC estável e com controles sem IC (Artigo I).

II. Avaliar a rigidez arterial e o acoplamento ventricular-arterial em pacientes com ICAD quando comparados a pacientes com IC compensada e controles sem IC (Artigo II).

III. Avaliar a atividade da matriz extracelular e depósito miocárdico de metaloproteinases em pacientes com diferentes formas de amiloidose cardíaca (Artigo III).

Artigo I

Episodes of Acute Heart Failure Syndrome are Associated with Accelerated Myocyte Loss and Increased Extracellular Matrix Turnover.

Episódios de Insuficiência Cardíaca Agudamente Descompensada estão Associados com Perda Acelerada de Cardiomiócitos e Aumento na Atividade da Matriz Extracelular.

Episodes of Acute Heart Failure Syndrome are Associated with Accelerated Myocyte Loss and Increased Extracellular Matrix Turnover

Short title: myocyte loss and ECM activation in AHFS

Word count: Abstract, 276; Total, 4625

Key Words: myocyte loss, extracellular matrix, acute decompensated heart failure

Abstract

Background: Increased myocyte loss and extracellular matrix (ECM) turnover are central mechanisms that contribute to pathologic myocardial remodeling in chronic heart failure (HF). We tested the hypothesis that episodes of acute heart failure syndrome (AHFS) are associated with transient further increases in myocyte loss and ECM turnover beyond those observed in chronic stable HF.

Methods and Results: Myocyte loss and ECM turnover were assessed in 80 patients prospectively divided into 3 groups: AHFS (n = 39); chronic stable systolic HF (n = 21); and control subjects without HF (n = 20). Myocyte death was assessed by measuring plasma troponin I. ECM turnover was assessed by measuring plasma matrix metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs), and pro-collagen N-terminal types I (PINP) and III (PIIINP). In the AHFS group, biomarkers were obtained a) at the time of hospital admission for an episode of HF decompensation, b) at the time of hospital discharge, and c) several weeks after discharge in patients who had returned to a chronic stable compensated state. In patients with stable HF (vs. non-HF controls), there was a small increase in troponin I, and little or no difference in any marker of ECM turnover. In patients with AHFS, troponin I and three markers of ECM turnover (MMP-2, TIMP-1 and PIIINP) were elevated (vs. chronic stable HF), and all fell toward chronic HF levels in patients who returned to a compensated state.

Conclusion: Episodes of AHFS are associated with transient increases in myocyte loss and ECM turnover that may accelerate pathologic myocardial remodeling, and thereby contribute to long term outcomes. These observations suggest a role for novel therapies that target myocyte loss and ECM turnover in AHFS.

Introduction

In reporting the consensus of the First and Second International Workshops on Acute Heart Failure Syndrome, Gheorghiade et al. ([1]) noted that episodes of acute heart failure syndrome (AHFS) have been considered an expected part of the chronic HF continuum, and consequently, "…failure to consider AHFS as a separate entity with distinct epidemiology and pathophysiology may have contributed to the slow progress of its recognition and management." ([1])

Pathological remodeling of the heart involves structural and functional abnormalities of cardiac myocytes and the extracellular matrix (ECM) that are mediated by several stimuli including mechanical strain, neurohormones and inflammatory cytokines ([2]). The importance of myocardial remodeling in the progression of chronic systolic HF is appreciated, and highlighted by the demonstration that therapies that inhibit myocardial remodeling, such as ACE inhibitors and beta blockers, ameliorate clinical outcomes including HF hospitalization and death ([3]).

Patients with chronic HF frequently experience episodes of AHFS that are characterized by interstitial fluid overload, elevated cardiac filling pressures, depressed cardiac output, and the attendant symptoms ([4]). It is not known whether processes central to pathological myocardial remodeling, such as myocyte loss and ECM turnover, are activated by episodes of decompensation. However, AHFS is associated with increased mechanical strain on the heart, activation of neurohormonal systems, and increased inflammation and oxidative stress ([5;6]), stimuli that are known to mediate myocardial loss and ECM turnover. Therefore, it is possible that myocyte

loss and ECM turnover are accelerated during episodes of AHFS.

To test this thesis, we prospectively measured circulating markers of myocyte death and ECM turnover in patients with a history of chronic systolic HF at the time of admission to the hospital for an episode of AHFS. We then used two strategies to establish a relationship between the episodes of AHFS and the biomarkers. First, we compared patients with AHFS to patients with chronic stable HF who had a similar level of myocardial dysfunction. Second, in a subgroup of the patients with AHFS, we measured the biomarkers sequentially as patients returned to a chronic compensated state early (days) and late (months) after the episode of AHFS.

Methods

Subjects. Three groups of subjects were prospectively recruited into this study: a) patients admitted to the hospital for treatment of AHFS, b) patients with chronic stable HF, and c) control subjects without HF.

Patients with AHFS were identified from patients admitted to Boston University Medical Center for an episode of systolic HF complicated by volume overload. Systolic HF was defined as a previous diagnosis of HF and echocardiographic demonstration of systolic left ventricular (LV) dysfunction with an ejection fraction (LVEF) < 45%. The diagnosis of AHFS with volume overload was defined clinically by worsening symptoms (dyspnea, paroxysmal nocturnal dyspnea, orthopnea) in the setting of clinical findings of circulatory congestion (elevated jugular venous pressure, hepatojugular reflux, hepatomegaly and/or peripheral edema). Patients with concomitant acute coronary syndromes

within the prior 3 months, primary infectious or inflammatory processes, or severe hemodynamic instability requiring intravenous vasoactive drugs were excluded. Also excluded were patients with aortic stenosis, malignancy, significant renal (creatinine > 3 mg/dL) or hepatic (cirrhosis or active hepatitis) dysfunction, and rheumatologic diseases.

Patients with chronic stable HF were recruited from the Cardiomyopathy Clinic at Boston University Medical Center. They had chronic stable symptoms, had not been hospitalized during the previous two months, were not volume-overloaded by clinical examination, and did not require a change in diuretic therapy on that visit.

Non-HF control subjects were recruited from ambulatory clinic or inpatient services at Boston University Medical Center. They had no history, symptoms or findings of HF. The research protocol was approved by the Institutional Review Board at the Boston University Medical Center. Written, informed consent was obtained from all participants.

Data and sample collection. For control subjects and stable HF patients, clinical data and blood samples were collected at a single time-point. Patients with AHFS had clinical data and blood samples obtained at least twice: a) during the first 24 hours of admission, b) and again just prior to hospital discharge. In a subset of the AHFS patients, blood samples were obtained a third time, late after discharge, if they met the criteria for chronic stable compensation with a) no evidence of volume overload by clinical examination, b) no need for diuretic adjustment, and c) no hospital admission for AHFS within the prior 2

months. A subjective dyspnea score was used to access symptom severity. Using an analog visual scale (0 = the most severe dyspnea ever experiences, and 100 = no dyspnea), the patients were asked to score their symptoms at the time of admission and again at the time of discharge.

Biomarkers. Blood samples were centrifuged and the plasma and serum were frozen at –70° C until the assays were performed. N-terminal proB-type natriuretic peptide (NT-proBNP) was analyzed in plasma using a commercially available ELISA kit (Alpco Diagnostics). Troponin I was measured in serum using a high sensitivity commercial chemiluminometric assay (ADVIA Centaur Ultra Troponin I; Siemens Medical Solutions Diagnostics). The detection threshold for this assay is 0.006 ng/mL, and abnormal levels are defined as values exceeding the 99th percentile of a reference control population (≥ 0.05ng/mL). Pro-collagen type I N-terminal peptide (PINP) and pro-collagen type III N-terminal peptides (PIIINP) levels were assessed in serum samples using a radioimmunoassay (Orion Diagnostica, Finland). Gelatinases (MMP-2 and -9) and tissue inhibitors of MMPs (TIMP-1, -2 and -4) were measured with commercially available ELISA kits (Amersham Pharmacia Biotech, Buckinghamshire, UK for MMPs and TIMP-1 and -2; R&D Systems Minneapolis, USA for TIMP-4). All specimens were processed in duplicate, and the mean intra-assay coefficient of variation was less than 7 % for all assays.

Statistical analysis. Continuous variables are expressed as mean ± standard deviation or median and interquartile (IQ) range, and categorical

variables are expressed as the number of patients or percentage. Comparisons among all groups for clinical variables were performed using ANOVA or chi-square tests as appropriate. Comparisons among groups for all biomarkers were performed using ANOVA and Tukey multiple-comparison post-hoc tests, or Kruskal-Wallis test for non-normally distributed variables. Troponin I values were also categorized according to the detectable and abnormal thresholds of the assay and analyzed using χ^2 statistics. Differences between admission and discharge values in the AHFS group we evaluated using paired *t* test or Wilcoxon rank test. The effect of medications on biomarkers was assessed by univariate, and followed by multivariate, regression analysis. Medications with significant effects were added to models with the respective biomarker as the dependent variable. A value of $p < 0.05$ was considered significant.

Results

Patient demographics and clinical characteristics (Table 1). A total of 80 subjects were included in this study as follows: AHFS = 39, chronic stable HF = 21, and non-HF control = 20. The groups were not different with regard to age or gender distribution; and had similar frequencies of diabetes, hypertension and ischemic heart disease. The use of HF medications was lower in non-HF controls, as expected, but similar in the AHFS and stable HF groups. Patients in the chronic stable HF group were predominantly in NYHA class I and II,

Table 1. Demographics and clinical characteristics.

	Controls (n=20)	Stable HF (n=21)	AHFS (n=39)
Age, years	54 ± 10	61 ± 14	61 ± 13
Sex, male/female	12/8	18/3	30/9
Body mass index, kg/m^2	30.7 ± 5.5	29.7 ± 7.6	30.8 ± 6.7
Creatinine	0.8 ± 0.2	1.2 ± 0.4	1.4 ± 0.6*
NYHA, I/II/III/IV	-	6/12/3/-	-/-/15/24†
NT-proBNP, fmol/mL	318 ± 116	664 ± 448	1869 ± 1345*†
Diabetes, %	35	48	38
Hypertension, %	60	71	77
Ischemic heart disease,	25	48	46
ACEi/ARB, %	35	90*	72*
Beta-blockers, %	50	95*	77*
Spironolactone, %	0	19	8

Values are means ± SD, number of patients or percentages. * $p < 0.05$ vs. Controls; † $p < 0.05$ vs. Sable Heart Failure. ACEi, Angiotensin Converting Enzyme inhibitors; ARB, Angiotensin II Receptor Blockers.

whereas patients with AHFS were all in NYHA class III or IV. Serum creatinine concentration was similar in the chronic stable HF and AHFS groups. NT-pro-BNP levels in patients with AHFS were markedly elevated, and higher than in chronic stable HF patients (**Figure 1A**).

Hemodynamic and echocardiographic characteristics (Table 2). LV size and function, although markedly abnormal, were similar in the chronic HF and AHFS groups. In both HF groups, the mean LV end-diastolic dimension was 59 - 60 mm, and the ejection fraction was 24%.

Table 2. Hemodynamic and echocardiographic characteristics.

	Controls (n=20)	Stable HF (n=21)	AHFS (n=39)
Systolic Blood Pressure, mmHg	128 ± 14	119 ± 26	123 ± 20
Diastolic Blood Pressure, mmHg	74 ± 8	71 ± 9	75 ± 13
Heart rate, bpm	69 ± 15	71 ± 13	77 ± 17
LV End-Diastolic Diameter, mm	47 ± 5	59 ± 7*	60 ± 10*
LV End-Systolic Diameter, mm	31 ± 3	48 ± 9*	50 ± 13*
LV Ejection Fraction, %	63 ± 3	24 ± 10*	24 ± 10*

Values are means ± SD, number of patients or percentages. * $p < 0.05$ vs. Control group; † $p < 0.05$ vs. Sable Heart Failure group. NYHA, New York Heart Association; LV, left ventricle.

Troponin I. The mean troponin I level was elevated in both HF groups (vs. non-HF controls), and was highest in the AHFS group (**Figure 1B**). Compared to non-HF controls, a higher proportion of patients in both HF groups had detectable troponin levels (>0.006 ng/mL): 91% of stable HF and 98% of AHFS group (vs. 53% of non-HF patients; $p <0.001$ for both). Likewise, troponin I levels ≥ 0.05 ng/mL, the institutional criteria for an abnormal elevation, were present in 27% of stable HF patients and 49% of AHFS patients, but none of the non-HF controls ($p<0.001$ vs. controls for both).

ECM markers. PINP and PIIINP reflect collagen synthesis. PIIINP was markedly increased in patients with AHFS, as compared to both stable HF patients and non-HF controls (**Figure 2A**), whereas PINP levels were similar in the 3 groups (**Fig 3A**). Neither PIIINP nor PINP was elevated in patients with stable HF, as compared to control subjects without HF.

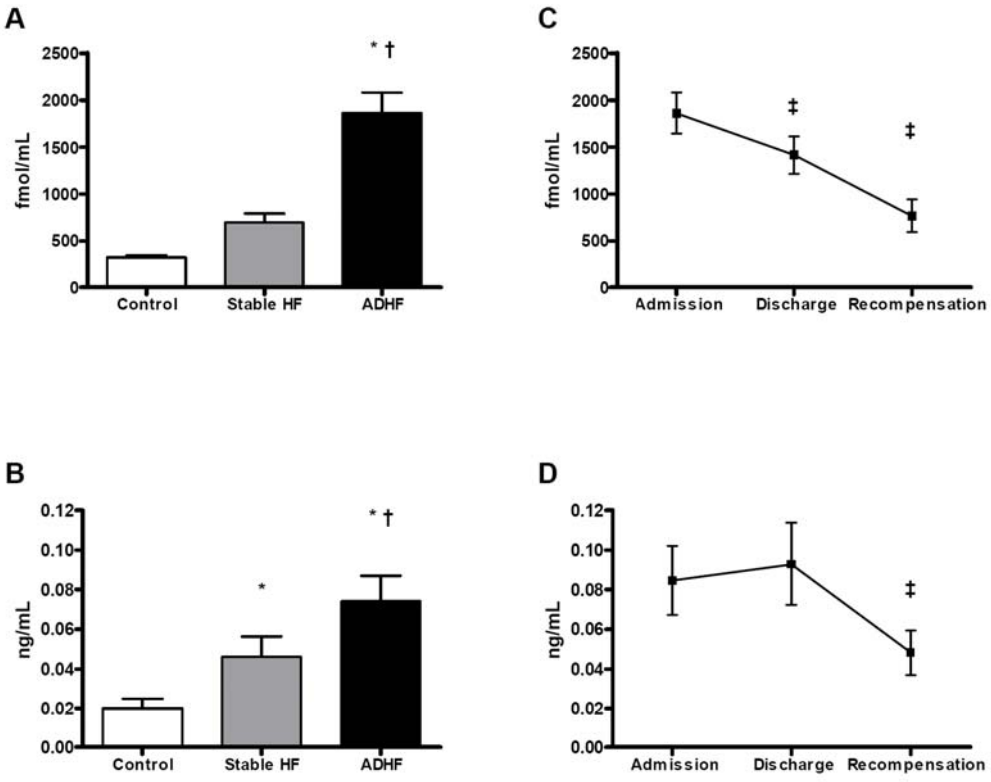

Figure 1. NT-proBNP and Troponin I results in the studied groups. A, NT-proBNP values in non-HF controls, stable HF and AHFS. B, troponin I values in non-HF controls, stable HF and AHFS. C, NT-proBNP levels in AHFS patients at admission, discharge and after chronic re-compensation. D, troponin I levels in AHFS patients at admission, discharge and after chronic re-compensation.

ECM degradation is regulated by MMPs and TIMPs. None of the MMPs or TIMPs was elevated in stable HF patients, as compared to non-HF controls. In patients with AHFS, MMP-2 and TIMP-1 levels were increased, as compared to patients with stable HF or non-HF controls (**Figures 2B and 2C**). In contrast, MMP-9 and TIMP-2 were not different in patients with AHFS vs. stable HF **(Figures 3B and 3C)**. TIMP-4 levels were increased in patients with

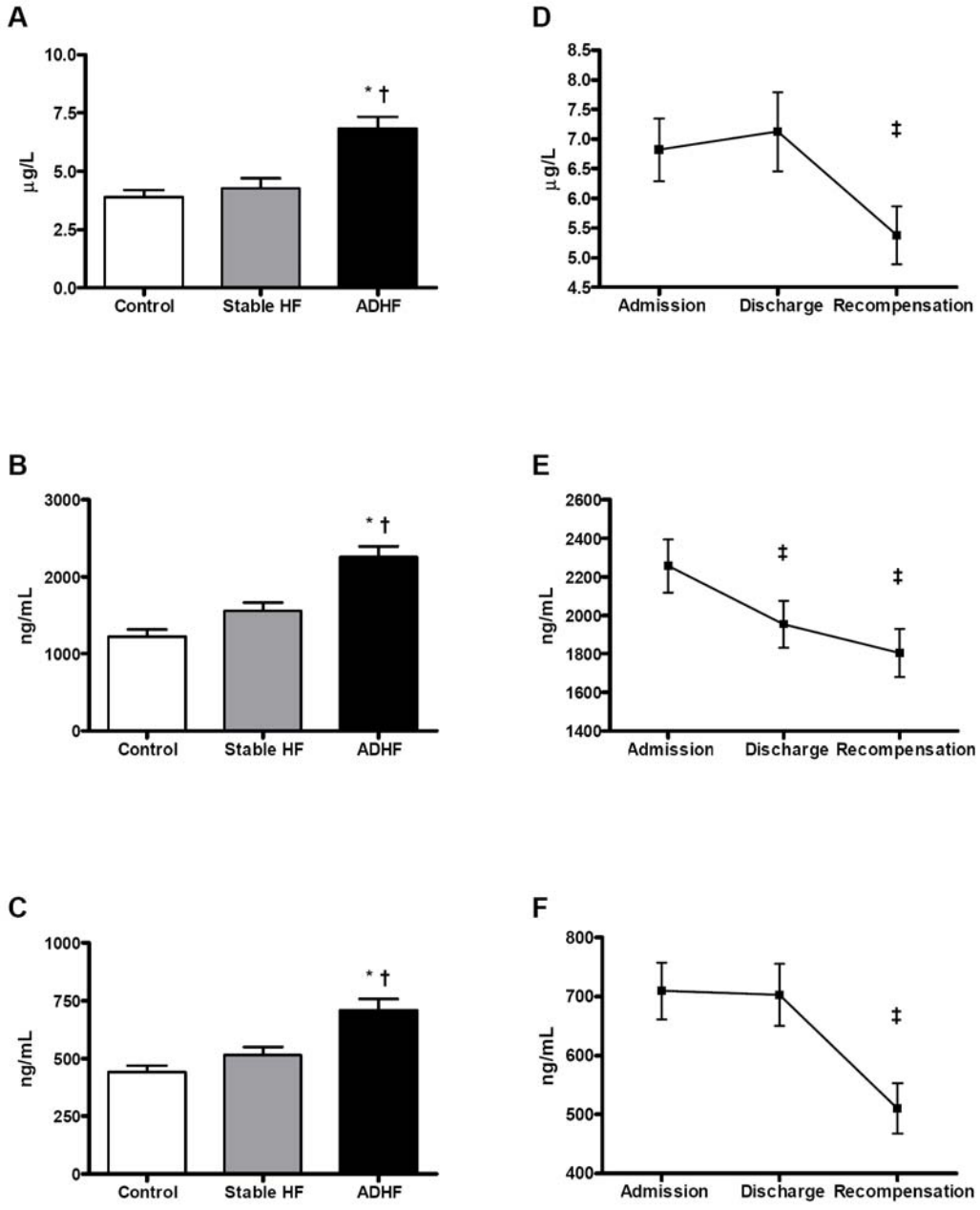

Figure 2. Extracellular matrix markers associated with AHFS. *Left,* PIIINP (A), MMP-2 (B) and TIMP-1 (C) values for non-HF controls, stable HF and AHFS patients. *Right,* PIIINP (D), MMP-2 (E) and TIMP-1 (F) values in AHFS patients at admission, discharge and after chronic re-compensation.

AHFS compared to non-HF controls, but were not different from patients with stable HF **(Figure 3D)**.

HF etiology. We evaluated whether the observed differences in biomarkers were related to the etiology of HF. Patients with ischemic HF represented 37% of HF patients (45% of stable HF and 34% of AHFS, p = 0.34). Troponin I levels tended (p = 0.19) to be higher in the patients with non-ischemic HF (0.046 ng/mL; IQ range, 0.025 - 0.087 ng/mL) compared to patients with an ischemic etiology (0.028 ng/mL; IQ range, 0.022 – 0.068 ng/mL). Likewise, all ECM markers were similar (p > 0.4 for all) in patients with ischemic vs. non-ischemic HF.

Medications. When patients were grouped by medications, there were no significant differences for troponin or any of the ECM markers with regard to use of ACEi/ARB, beta-blockers or spironolactone. Likewise, controlling for ACEi/ARB or beta-blocker use had no effect on the inter-group differences observed for troponin and ECM markers (data not shown).

Effect of acute therapy. For the patients with AHFS, the duration of hospital admission averaged 5 ± 6 days. The predominant treatment during the admission was the use of intravenous diuretics leading to an average weight loss of 3.6 ± 0.7 kg. This diuresis was associated with a marked improvement in HF symptoms as reflected by an increase in the dyspnea subjective score from 33 to 85 (p<0.0001). Likewise, NT-proBNP decreased from 1869 ± 1345 fmol/mL to 1415 ± 1160 fmol/mL over the course of the admission (p< 0.05) **(Figure 1C)**.

Troponin I values were unchanged at the time of discharge (0.055 ng/mL; IQ range, 0.027 - 0.1

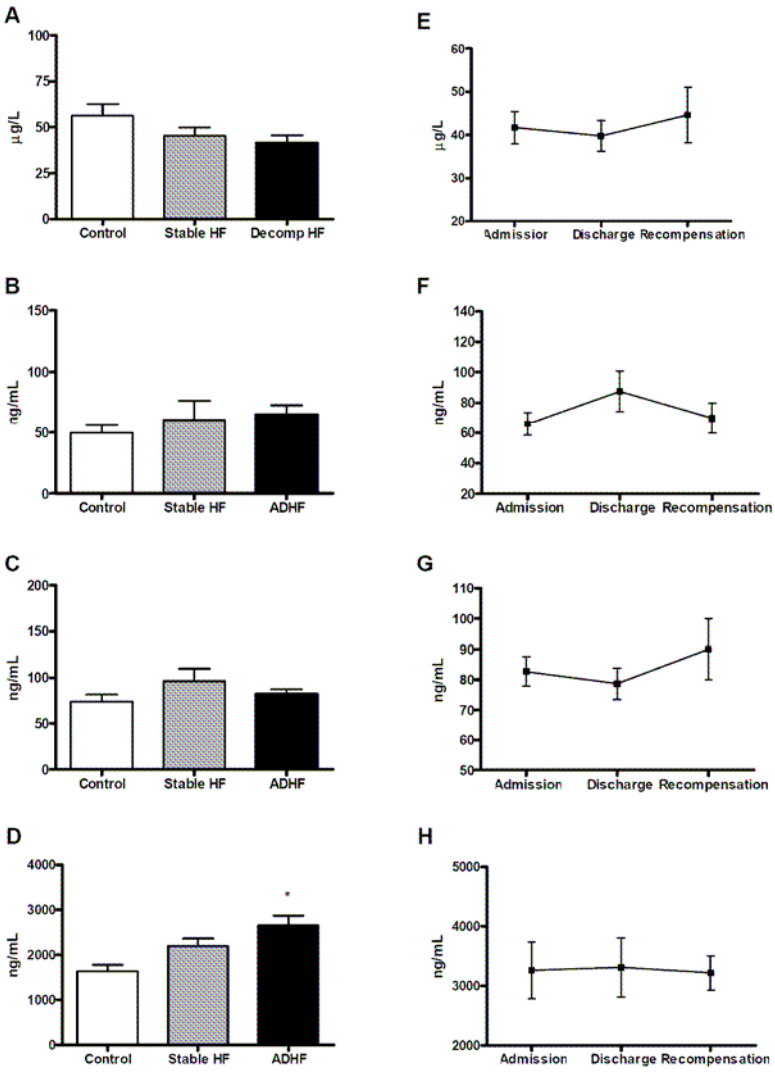

Figure 3. Extracellular matrix markers not associated with AHFS. *Left*, PINP (A), MMP-9 (B), TIMP-2 (C), and TIMP-4 (D) values for non-HF controls, stable HF and AHFS patients. *Right*, PINP (E), MMP-9 (F), TIMP-2 (G), and TIMP-4 (H) values in AHFS patients at admission, discharge and after chronic re-compensation.

ng/mL) as compared admission (0.046 ng/mL; IQ range, 0.028 – 0.08 ng/mL, p = 0.77) **(Figure 1D)**. Among the ECM markers, MMP-2 was decreased at discharge, whereas PIIINP and TIMP-1 remained elevated **(Figure 2D-F)**.

Chronic compensation. In a subgroup of 16 AHFS patients who subsequently met the criteria for chronic stable HF (see Methods),

biomarkers were measured again an average of 8 ± 3 months after discharge. NT-proBNP levels returned to values similar to the stable HF group **(Figure 1C)**. Compared to levels observed during decompensation, both troponin I (**Figure 1D**) and all 3 of the ECM markers that were elevated with AHFS (PIIINP, MMP-2 and TIMP-1) were decreased (**Figure 2D-F**). In contrast, PINP, MMP-9 and TIMP-2, which were not increased with AHFS, remained unchanged from admission values (**Figure 3E-H**).

Discussion

The major finding of this study is that episodes of AHFS are associated with transient increases in the blood levels of troponin I, a marker for cardiac myocyte death, and three markers for ECM turnover (MMP-2, TIMP-1 and PIIINP). Compared to patients with chronic stable HF, troponin I and these ECM markers were elevated in patients with AHFS. Of note, when AHFS patients returned to chronic stable HF, all of the elevated markers returned to or towards the levels observed in the chronic stable HF group. In contrast, patients with stable HF had only a modest increase of troponin I levels above that in non-HF controls, and little or no alteration in any of the ECM markers.

Troponin, a marker of myocyte death, is known to be elevated in patients with HF in the absence of epicardial coronary artery disease ([7;8]). In patients with HF, elevated troponin levels are associated with a worse prognosis ([8-12]). While troponin I is highly specific for cardiac myocyte death, circulating levels may also be elevated due to renal insufficiency. However, this does not appear to underlie our observations, since serum creatinine levels were similar in the chronic stable HF and AHFS groups (see Table 1). Furthermore, there was no relationship between troponin and creatinine levels within the AHFS group. Thus, we believe that the transient elevation in circulating troponin I in our

AHFS patients reflects increased myocyte death.

Myocyte loss is believed to play an important role in adverse myocardial remodeling and the progression of HF, regardless of the etiology ([2]). Myocyte death may be due to necrosis or apoptosis, both of which may result in the leakage of troponin. A common cause of cardiac myocyte death is ischemia due to coronary artery disease. However, troponin levels in our study were similar in patients with non-ischemic and ischemic HF, and actually tended to be higher in the non-ischemic group. While our data do not allow us to determine the cause of increased myocyte death during AHFS, these episodes are associated with processes that are known to cause myocyte death including mechanical strain, oxidative stress and neurohormonal activation ([13-16]).

Quantitative and qualitative alterations in the composition of the cardiac ECM are another important component of pathologic myocardial remodeling ([2]). ECM composition is determined by the balance of degradative and synthetic processes, and accordingly, circulating levels of MMPs, TIMPs and collagen fragments have proved useful in providing evidence of increased ECM turnover in patients with HF ([17-24]). In cross-sectional studies of patients with systolic HF, alterations in circulating MMP and TIMP levels are related to the extent of LV remodeling and predict clinical outcomes ([17;23;24]), thus supporting the clinical relevance of these biomarkers.

No prior study has assessed the relationship of ECM turnover to episodes of AHFS, nor is it known whether ECM turnover is increased during an episode of AHFS. In this

regard, our study provides two new observations about ECM turnover in HF. First, markers of ECM turnover were not increased in our patients with chronic stable HF. Second, during episodes of AHFS there were marked increases in three markers of ECM turnover (MMP-2, TIMP-1 and PIIINP). The relationship of these biomarkers to AHFS was supported by the sequential demonstration, in a subgroup of AHFS patients, that these markers returned to or toward the levels observed in compensated patients with chronic stable HF. The transient elevations in 3 ECM markers do not appear to be related to altered renal clearance, for two reasons. First, renal function was similar in the chronic stable and AHFS groups. Second, other structurally similar ECM markers (i.e., PINP, MMP-9, TIMP-2 and TIMP-4) were not elevated in the AHSF group.

We believe that our ability to identify differences in troponin and ECM biomarkers in our patients with AHFS, as compared to those with chronic stable HF, reflects two relatively unique aspects of our study design. First, we prospectively grouped our patients as compensated (i.e., chronic stable) or decompensated (i.e., AHFS requiring admission to the hospital). In this regard it is noteworthy that, while the chronic stable and decompensated HF groups had identical degrees of LV dilation and systolic dysfunction, they differed markedly with regard to symptom severity, NYHA functional class and BNP levels. Prior studies have generally examined patients with heterogeneous or ill-defined levels of clinical stability and compensation. A second important feature of our design is the use of a non-HF control group that had a similar incidence of concomitant cardiovascular

risk factors and conditions that are associated with ECM turnover, including hypertension ([25]), diabetes ([26]) and coronary artery disease ([27]).

A limitation of this study is that circulating ECM markers may not reflect events in the myocardial matrix. Although circulating levels of ECM components have been shown to relate to myocardial levels ([22;23]), we can not exclude the possibility that there are contributions from the vasculature and other organs. Another limitation is the relatively small number of patients, which may have decreased our ability to detect small changes in ECM markers in the stable HF group, and to correct completely for factors that affect these biomarkers. However, the number of patients was adequate to detect increased troponin levels in the stable group, and thus it is unlikely that an important difference in ECM biomarkers was missed in that group. Finally, these data do not allow conclusions regarding the mechanism responsible for increased myocyte loss and ECM turnover. Neurohormones, inflammation and oxidative stress present in AHFS are known to regulate both processes ([28-30]). Further studies are necessary to address the role of these and other factors.

When the symptoms of AHFS are sufficient to preclude ambulatory management, patients are admitted to the hospital for intensive therapy that focuses on fluid removal, and in some cases, the bolstering of hemodynamic function with vasodilators and positive inotropic agents. The primary goal of therapy is to alleviate symptoms and restore the compensated state. Ongoing cardiac myocyte death due to necrosis and/or apoptosis, and qualitative and quantitative changes in ECM

composition are central mechanisms in myocardial remodeling. Accordingly, an important implication of our observations is that episodes of decompensation may be associated with an acceleration of pathological myocardial remodeling. We agree with Gheorghiade et al. ([1]), who suggested that "…. should further research establish the presence and magnitude of myocardial injury in AHFS, preventing or limiting it with acute interventions may result in improvement in long-term outcome." We believe that the present study provides some of the first evidence that AHFS is associated with transient increases in both myocyte death and ECM turnover. As such, these observations have implications regarding the importance of preventing episodes of AHDF and the identification of therapeutic targets related to cell death and ECM turnover in this setting.

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Disclosures

None.

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Artigo II

Increased Arterial Stiffness Contributes to Vascular Load in Acute Decompensated Heart Failure.

Rigidez Arterial Aumentada Contribui para a Pós-Carga Vascular na Insuficiência Cardíaca Agudamente Descompensada.

Increased Arterial Stiffness Contributes to Vascular Load in Acute Decompensated Heart Failure

Short title: arterial stiffness in acute decompensated heart failure

Word count: Abstract, 248; Total, 4253

Key Words: arterial stiffness, vascular load, acute decompensated heart failure

Abstract

Background: Vascular load is an important determinant of left ventricular (LV) function, and comprises two major components: resistive load, and pulsatile load due to increased arterial stiffness. It is not known if arterial stiffness and pulsatile load are abnormal in acute decompensated heart failure (ADHF). We sought to investigate if arterial stiffness is increased in ADHF and is reduced by clinically effective therapy.

Methods: We studied a) 20 outpatients with chronic stable HF, b) 24 patients admitted for ADHF, and c) 20 controls without HF. Arterial stiffness was assessed by carotid-radial (CR) and carotid-femoral (CF) pulse wave velocity using applanation tonometry. Systemic vascular resistance index (SVRi, a measure of resistive load), arterial elastance (Ea, a measure of global vascular load), and LV stroke work index (LVSWi), were assessed using ultrasound-derived stroke-volume. In the ADHF group, studies were performed within 24 hours of admission and repeated prior to discharge.

Results: In ADHF, peripheral arterial stiffness (CR), resistive load (SVRi) and global vascular load (Ea) were all increased, with a parallel decrease in LVSWi. CR, SVRi and Ea were not different in stable HF vs. controls. After treatment in the ADHF group (predominantly diuresis), CR, SVRi and Ea all decreased while LVSWi increased.

Conclusion: Arterial stiffness is increased in ADHF in concert with increased resistive load and global vascular load; all decreased with effective treatment with

a concomitant improvement in LV performance. Increased arterial stiffness and pulsatile load may contribute to the pathophysiology of ADHF and may have therapeutic implications.

Introduction

Increased arterial stiffness, a hallmark of the aging process and the consequence of many disease states, is an important risk factor for cardiovascular disease, including myocardial infarction, heart failure (HF), and total mortality([1-4]). An increase in arterial load is a key element in the pathophysiology of many cardiovascular diseases including HF([5]). The arterial system serves two important functions: as an elastic buffer, damping pulsatile, high-pressure flow in the proximal aorta into low pressure, non-pulsatile flow by the time the pulse wave reaches the resistance vessels and capillary bed, and as a conduit allowing transmission of blood flow to the periphery. The failing ventricle is exquisitely sensitive to changes in pressure and vascular load; subtle abnormalities in either the damping or conduit functions of the arterial system may result in a disproportionately large fall in cardiac output. Vascular load is composed of two components – the resistive load generated by resistance vessels at the level of the arterioles, and the pulsatile load imposed by increased arterial stiffness and wave reflection in conduit arteries. Increased arterial stiffness significantly increases pulsatile and hence vascular load, adversely impacts ventricular-vascular coupling and increases the load on the left ventricle([6]). Changes in arterial properties (increased stiffness and pulsatile load, or reduced compliance) have previously been demonstrated in patients with

stable HF([7-10]). The underlying mechanisms for increased arterial stiffness in HF may include structural alterations in the extracellular matrix, neurohormonal activation, inflammation and increased oxidative stress, as well as vascular wall edema([5;11;12]). It has been suggested that abnormal pulsatile load may contribute to the pathophysiology of HF([10;13]).

There are now over a million admissions for acute decompensated HF (ADHF) a year in the United States, representing a substantial proportion of total healthcare expenditure on HF([14]). Furthermore, these episodes of decompensation may contribute to the progression of HF syndromes([15;16]). In a milieu of neurohormonal activation and increased load, the contribution of the arterial system may be an important factor in determining ventricular work efficiency and response to therapy([17]). However, understanding of the pathophysiology of ADHF remains incomplete, and the contribution of increased arterial stiffness is unknown. Better understanding of this syndrome would allow the development of more effective therapeutic strategies which might not only improve symptoms but also slow the progression of chronic HF.

We therefore hypothesized that arterial stiffness is increased in ADHF and contributes to total vascular load. The goals of this study were to evaluate arterial stiffness and vascular load in a population of patients with ADHF, and to assess the effects of clinically effective therapy for ADHF on arterial

stiffness, load and ventricular performance.

Methods

Study Subjects

Two groups of patients with systolic HF (left-ventricular ejection fraction [LVEF] < 45%) were recruited: 1) patients hospitalized for ADHF and 2) patients with stable HF. ADHF was defined as the presence of suggestive symptoms (worsening dyspnea, dyspnea at rest, paroxysmal nocturnal dyspnea and orthopnea) with physical findings (increased jugular venous pressure, hepato-jugular reflux, hepatomegaly, pulmonary rales and peripheral edema) and/or objective findings (chest X-ray findings, elevated serum B-type natriuretic peptide levels) consistent with ADHF. Patients with ADHF were identified through screening of hospital admission lists and recruited within 24 hours of admission. Patients with hemodynamic instability or with suspected or confirmed acute coronary syndrome were not included. Stable HF was defined as stable symptoms without hospitalization during the previous two months, without volume-overloaded on clinical examination, and if no changes in therapy were required. These patients were recruited from Boston University Medical Center Cardiomyopathy and Cardiology clinics. Exclusion criteria for all groups included acute coronary syndrome within the previous 3 months, atrial fibrillation, uncontrolled hypertension (systolic blood pressure > 180 mmHg, diastolic blood pressure > 100 mmHg), significant carotid or

peripheral vascular disease, significant acute infection, severe left ventricular outflow obstruction, active malignancy, significant renal or hepatic dysfunction, or systemic inflammatory states. Control subjects without previous history, symptoms or clinical findings of HF were identified through screening of Cardiology and Internal Medicine clinic lists and from admission logs of patients admitted for non-cardiac causes at Boston University Medical Center. The study protocol was approved by the institutional review board and all subjects gave informed consent.

Data collection

Clinical data and blood samples were collected for all subjects at enrollment. For patients with ADHF, the clinical data and blood samples were obtained again at the day of hospital discharge. A dyspnea subjective score was used to access symptom severity in patients with ADHF: using an analog visual scale where 0 represents the most severe dyspnea ever felt and 100 represents no dyspnea, the patients reported their symptoms at the time of the initial evaluation and again prior to discharge. Blood samples were centrifuged and the plasma and serum were frozen at –70°C until analyses were performed.

For the assessment of arterial properties, subjects were studied in the supine position after 5 min of rest. Blood pressure was measured manually twice or until 2 sequential readings differing by less than 5 mmHg were obtained. Arterial tonometry with simultaneous ECG was obtained at the radial, femoral

and carotid arteries using a commercially available tonometer (Sphygmocor, Atcor Medical). The body surface distances from the suprasternal notch to the carotid (SSN-C), radial (SSN-R) and femoral (SSN-F) recording sites were measured using a tape measure, and the adjusted distances between recording sites were calculated by subtracting SSN-C from SSN-R and SSN-F. The delay between the appearance of the foot of the pressure waveform in the carotid and the respective peripheral sites was then recorded. The adjusted distances were then divided by the respective foot-to-foot transmission delays to generate carotid-femoral (PWV-CF) and carotid-radial (PWV-CR) pulse wave velocities.

Subjects were then placed in the left lateral decubitus position, and echocardiographic images of the left ventricular outflow tract (LVOT) were obtained from a parasternal long axis view. Finally, pulsed Doppler of the LVOT was acquired from an apical 5-chamber view and velocity-time integral (VTI) was obtained. Stroke volume (SV) was determined as the product of aortic cross-sectional area (CSA) and VTI([18]). Aortic CSA was calculated by circular geometry [CSA = $(LVOT/2)^2$*3.14)] Effective arterial elastance (Ea) was determined as a measure of total vascular load([19]). Ea is equal to the ratio of end-systolic pressure (P_{es}) to stroke volume (SV). P_{es} may be accurately estimated by 0.9·arterial systolic pressure([19]). Left ventricular systolic work index (LVSWi) was calculated as the product of SV, indexed to BSA, and mean arterial pressure, and was converted into gram-meters

by multiplying by 0.0136. Finally, systemic vascular resistance index (SVRi) was estimated as [(mean arterial pressure/cardiac index)*80].

Data Analysis

Comparisons between groups were performed using ANOVA with Tukey post-hoc comparison for continuous variables, and χ^2 statistics for dichotomous variables. For main arterial measurements, multivariable analysis was performed and statistical models were constructed using general linear model adjusting for relevant covariates. For the ADHF group, comparison of the change between admission and discharge values were performed using paired *t*-test. Values are presented as mean ± SEM for continuous variables and as a number (percentage) for dichotomous variables, except where noted. A two-sided *p* value lower than 0.05 was considered statistically significant.

Results

Clinical characteristics

A total of 64 patients were included in this study: 24 with ADHF, 20 patients with stable HF and 20 non-HF controls. Table 1 provides the baseline clinical characteristics of the 3 groups. There were no significant differences between the three groups with regards to baseline characteristics, apart from a lower prevalence of dyslipidemia in the control group. While use of diuretics was slightly higher, and beta-blocker,

Table 1. Demographics, risk factor profile and cardiovascular medications

	Controls (n=20)	Stable HF (n=20)	ADHF (n=24)
Age, years	54 ± 10	61 ± 11	60 ± 12
Gender, male	12 (60)	18 (90)	19 (79)
Race, white	13 (65)	9 (45)	9 (37)
BMI, kg/m2	30.7 ± 5.5	29.7 ± 7.5	29.5 ± 7.4
Coronary artery disease	5 (25)	10 (50)	11 (46)
Hypertension	12 (60)	16 (80)	18 (75)
Diabetes	7 (35)	12 (60)	11 (46)
Dyslipidemia	7 (35)*	15 (75)	14 (58)
Beta-blockers	10 (50)*	20 (100)	19 (79)
ACEi/ARB	7 (35)*	18 (90)	18 (75)
Diuretics	4 (20)*	17 (85)	24 (100)
Statin	8 (40)	15 (75)	10 (42)
Spironolactone	0	4 (20)	0
Isosorbide	1 (5)*	5 (25)	8 (33)
Hydralazine	0*	6 (30)	5 (21)

* $p < 0.05$ for controls vs. other groups

ACE inhibitor and angiotensin receptor blocker use lower in the ADHF group compared to stable HF, the difference between the two groups was not statistically significant. As expected, medication use in the HF groups was significantly different compared to the control group,

Table 2 provides clinical information related to the HF groups. As expected, symptoms of HF were more severe in the ADHF group with a greater proportion of patients being

Table 2. Clinical, hemodynamic and structural/functional data related to heart failure

	Controls (n=20)	Stable HF (n=20)	Decompensated HF (n=24)
Ischemic etiology	-	50%	38%
NYHA functional class (I/II/III/IV)	-	6/13/1/0	0/1/6/17‡
Systolic blood pressure, mmHg	128 ± 14	117 ± 24	129 ± 18
Diastolic blood pressure, mmHg	74 ± 8	71 ± 9	79 ± 11*
Pulse pressure, mmHg	55 ± 10	47 ± 20	50 ± 15
Heart rate, bpm	69 ± 15	66 ± 10	76 ± 16*
LV End-diastolic diameter, mm	47 ± 5	60 ± 6†	59 ± 11†
LV End-systolic diameter, mm	31 ± 3	49 ± 9†	48 ± 13†
LV ejection fraction, %	63 ± 3	23 ± 10†	24 ± 12†
NT-proBNP, fmol/mL	318 ± 116	688 ± 396	1729 ± 1159†‡

NT-proBNP : N-terminal prohormone brain natriuretic peptide

* p = 0.05 as compared to Stable HF group; † p < 0.01 as compared to Control group; ‡ p < 0.01 as compared to Stable HF group.

in NYHA functional classes III and IV. Brachial diastolic blood pressure and heart rate were higher in the ADHF group. Parameters of LV size and function were similar in the ADHF and stable HF groups. Values of NT-proBNP were higher in the ADHF compared to the other two groups.

Arterial properties

Main arterial properties are shown in Figure 1. Subjects with stable HF had similar values of CF-PWV and CR-PWV compared to

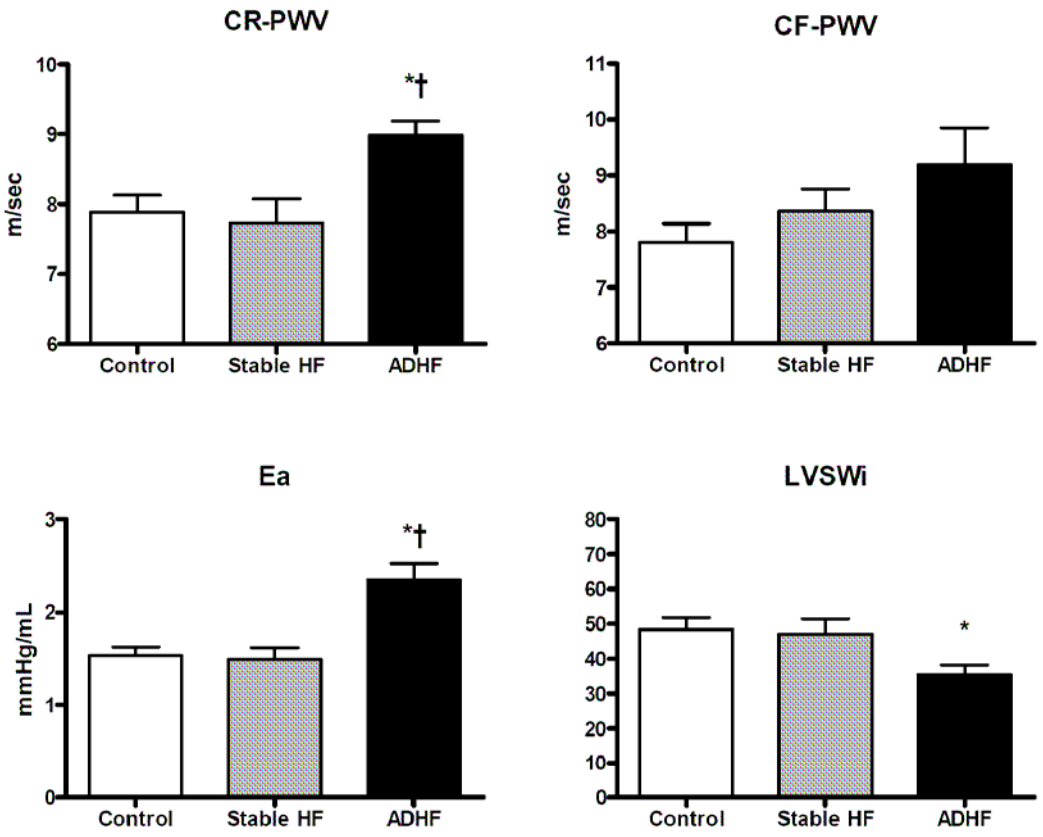

Figure 1. Arterial properties (Carotid-radial PWV, Carotid-femoral PWV and Arterial elastance, Ea) and LV stroke work index (LVSWi) in controls, patients with stable (Stable HF) and acute decompensated HF (ADHF). * $p < 0.05$ vs. controls; † $p < 0.05$ vs. Stable HF.

controls, suggesting arterial stiffness was unaltered in patients with stable HF. However, CR-PWV was significantly higher in patients with ADHF compared to stable HF and controls, with a similar, although non-significant increase in CF-PWV. This is consistent with an increase in the stiffness of more distal (muscular) conduit arteries and to a lesser extent, of more proximal conduit arteries. The systemic vascular

resistance index was significantly elevated in patients with ADHF (4031 ± 1179) compared to the other two groups (2974 ± 642 for stable HF and 3083 ± 677 for controls), suggesting the resistive component of load was also increased. Total vascular load, as indexed by Ea, was similarly increased in ADHF compared to stable HF and control subjects. LV stroke work index was lower in the ADHF group, in parallel with the increase in arterial stiffness and total vascular load. The arterial pulse pressure was similar in all three groups (Table 2).

As age, body mass index, heart rate, and blood pressure are known to affect measures of arterial stiffness and vascular load, we constructed multivariate regression models to adjust for the influence of those covariates. Furthermore, we evaluated whether medication use (ACEi/ARBs, beta-blockers, and vasodilators) was associated with the arterial properties. Beta-blocker use was associated to lower CR-PWV and Ea, and none of the other medications were associated to arterial properties. We therefore included beta-blocker use in the multivariate models. Table 3 shows the multivariate models for CR-PWV and Ea; both remained strongly associated with ADHF after adjustment for covariates. ADHF and beta-blocker therapy were the only significant determinants of CR-PWV values. Heart rate, systolic blood pressure and body mass index were other significant predictors of Ea in the multivariate model.

Table 3. Multivariate models showing arterial properties in Stable and Decompensated groups adjusted for covariates

	B	SE	*p* value
Carotid-Radial PWV			
Age, y	-0.018	0.015	0.216
Systolic blood pressure, mmHg	0.008	0.008	0.361
Heart rate, bpm	0.011	0.011	0.6322
Body mass index, kg/m^2	0.013	0.023	0.578
Beta-blocker therapy	0.194	0.060	**0.002**
Stable HF	-0.058	0.392	0.881
Decompensated HF	1.380	0.393	**0.001**
Arterial Elastance			
Age, y	-0.006	0.008	0.436
Systolic blood pressure, mmHg	0.008	0.004	**0.044**
Heart rate, bpm	0.017	0.005	**0.003**
Body mass index, kg/m^2	-0.027	0.011	**0.021**
Beta-blocker therapy	0.007	0.029	0.820
Stable HF	0.155	0.193	0.425
Decompensated HF	0.677	0.200	**0.002**

B: regression coefficient; SE: standard error of B; PWV: pulse wave velocity.

Clinical course during admission

During a median period of 4.5 (range 2-11) days between the initial and pre-discharge studies, patients in the ADHF group had a mean weight loss of 3.0 ± 2.4 kg and the subjective dyspnea score improved from 28.4 to 85.5 (Table 4). Systolic and diastolic blood pressures were lower at the pre-discharge study compared to the initial study, while pulse pressure was unchanged. NT-

Table 4. Clinical and hemodynamic parameters in the ADHF group: admission vs. discharge

	Admission	Discharge	*p* value
Systolic blood pressure, mmHg	128 ± 19	119 ± 18	0.001
Diastolic blood pressure, mmHg	79 ± 11	71 ± 11	0.001
Pulse pressure, mmHg	49 ± 13	47 ± 15	0.47
Heart rate, bpm	78 ± 16	75 ± 13	0.36
Weight, kg	92.7 ± 31.2	89.7 ± 31.7	<0.001
Subjective dyspnea score, %	28.4 ± 20.5	85.5 ± 13	< 0.001
NT-proBNP, fmol/mL	1794 ± 1200	1432 ± 1241	0.008

NT-proBNP : N-terminal prohormone brain natriuretic peptide

proBNP levels were elevated at admission and fell prior to discharge. During hospitalization, a beta-blocker was started in 2 of the 24 patients, and dosage was increased in 3 patients previously on beta-blockers. Angiotensin-converting enzyme inhibitors (ACEi) or angiotensin-receptor blocker (ARB) was started in 1 patient during the admission and dosage was increased in 5 patients. As expected, the predominant intervention in this group of patients was diuretic therapy for improvement of the congestive state.

Changes in arterial properties and hemodynamic values with clinical therapy

Changes in arterial properties and vascular load following effective treatment of HF are shown in Figure 2. There was a significant decrease in CR-PWV, CF-PWV, and Ea between the initial and pre-discharge studies. SVRi was also reduced from

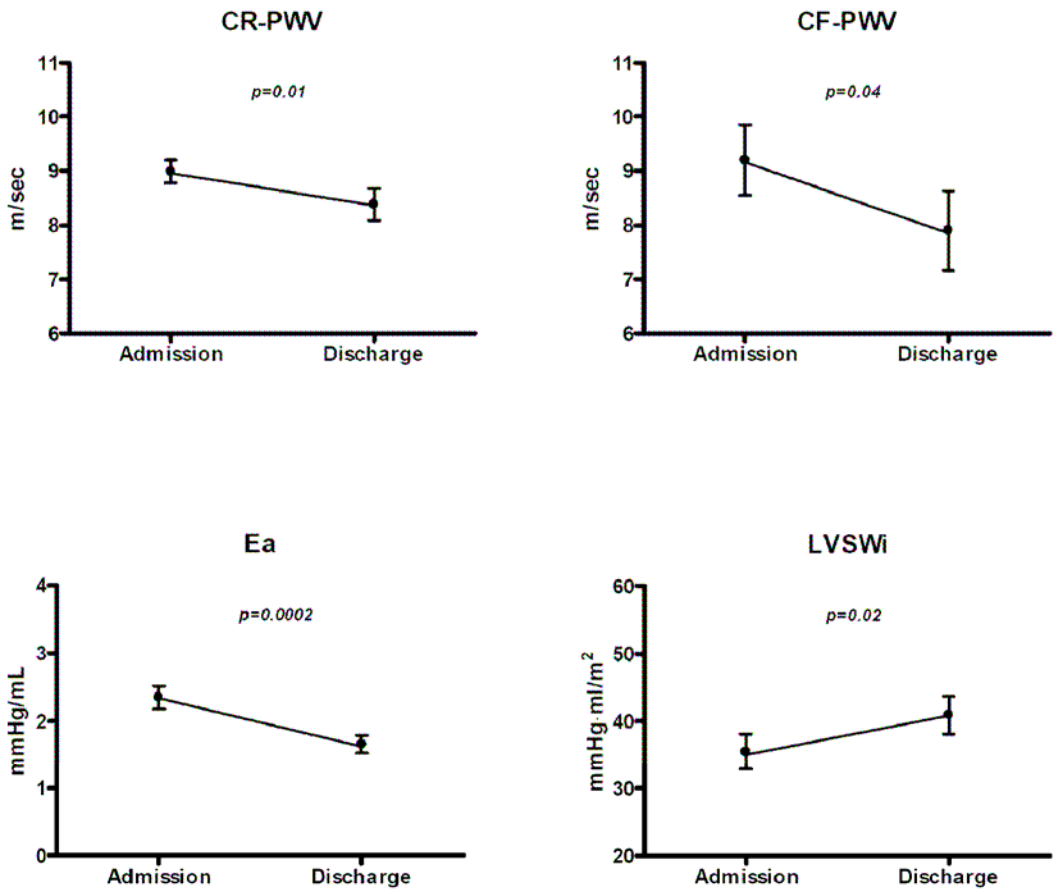

Figure 2. Arterial properties (Carotid-radial PWV, Carotid-femoral PWV and Arterial elastance, Ea) and LV stroke work index (LVSWi) in patients with ADHF: admission and discharge values.

admission (4013 ± 1317) to discharge (3126 ± 890, p = 0.001). LV stroke work index showed a parallel increase from admission to discharge, suggesting that the decrease in arterial stiffness, resistance and load may contribute to the improvement in cardiac performance. Again, pulse pressure did not change form admission (49 ± 13) to discharge (47 ± 15, p = 0.47).

Discussion

The main findings of the present study are that arterial stiffness, resistive load, and total arterial load 1) are increased during episodes of ADHF, and 2) are reduced concomitant with effective therapy, primarily with diuretics. Increased arterial stiffness and pulsatile load may contribute to the pathophysiology of ADHF.

Increased arterial stiffness has several consequences to the failing heart: the load imposed to the ventricle is higher, the efficiency of the cardiac ejection is lower, and the perfusion of the heart itself is reduced. Previous studies demonstrated reduced aortic compliance([20]) and increased wave reflection from the periphery([8]) in patients with stable, compensated HF. However, Mitchell and colleagues reported contrasting changes in central and peripheral stiffness in patients with stable, chronic HF: increased characteristic impedance (proximal stiffness), no changes in carotid-femoral PWV (central aortic stiffness), and reduced carotid-radial PWV (distal muscular artery stiffness)([10]). In fact, ventriculoarterial coupling has been shown to be abnormal in patients with systolic HF([21]), with increased arterial load (Ea) and decreased LV contractile function (Ees), resulting in a substantially increased Ea/Ees ratio and a greater dependence of the ventricle on the arterial load. Taken together, these findings suggest a significant pathophysiologic role for increased arterial stiffness and abnormal

ventriculoarterial coupling in systolic HF.

Our study is the first to demonstrate transient changes in arterial stiffness during episodes of ADHF. A possible mechanism underlying this finding is that the vascular alterations associated to stiffening are influenced by hemodynamic forces, as well as by extrinsic factors such as neurohormones and salt([5]). In support of this mechanism, carotid wall thickness and stiffness are increased in patients with systolic HF and correlate with plasma norepinephrine and aldosterone levels([9]). Also, a relationship between sympathetic nerve activation and arterial stiffness has been shown([22]). Interestingly, patients with ADHF had increased distal conduit arteries stiffness, with less evident changes on central stiffness, contrasting to findings in chronic stable HF. The stiffness component on peripheral arteries may become more important during episodes of decompensation. Both increased vascular sodium content and increased tissue pressure related to edema, especially in muscles surrounded by poorly compliant fascia, are possible explanations for this peripheral arteries stiffening([23;24]).

Blood pressure measured at the brachial artery is the simplest but crudest measure of vascular load, and it is imperfect due to confounding factors that typically underestimates total vascular load. Despite that, elevated pulse pressure, an indicator of increased arterial stiffness, has been shown to predict the development of

cardiovascular disease and HF([25;26]). Furthermore, higher pulse pressure is associated to adverse outcomes in patients with asymptomatic left ventricular dysfunction and HF([27;28]). However, pulse pressure is determined by the interaction of several hemodynamic factors including stroke volume and aortic blood flow, and therefore its relation to arterial stiffness may change as HF progresses to a decompensated state. In fact, the association of pulse pressure to outcome in ADHF is reverse, with low pulse pressure being a predictor of higher mortality([29]). In our study, pulse pressure failed to show both the increase in arterial stiffness with decompensation and its decrease after therapy.

Some limitations of our study must be acknowledged. Our sample size is relatively small; however, we selected controls without HF with several other risk factors to make it possible to attribute the differences to HF and not to other associated risk factors. Also, patients with stable HF and ADHF were similar regarding to structural disease and medications. Furthermore, the follow-up of decompensated patients strengthens the association of increased arterial stiffness to the decompensation episode. However, we are not able to determine which therapy was responsible for decreased stiffness and arterial load with effective therapy. We believe diuresis, the main therapy, might be implicated, but further controlled studies must be performed to address that. Finally, we acknowledge that the assessment of arterial load and stiffness and

ventriculoarterial interaction without using invasive measures of pressure and volume is limited and must accept some assumptions.

In conclusion, arterial stiffness is increased in ADHF in concert with increased resistive load and global vascular load; all decreased with effective treatment of the decompensation episode. The increased arterial stiffness during ADHF may have important clinical implications: first, vascular load due to increased arterial stiffness may adversely afftect ventricular function during episodes of decompensation; second, therapeutic strategies directed at “destiffening” central and peripheral arteries might have a significant role in the management of patients with ADHF.

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Disclosures

None.

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Artigo III

Matrix Metalloproteinases and their Tissue Inhibitors in Cardiac Amyloidosis: Relationship to Structural, Functional Myocardial Changes and to Light Chain Amyloid Deposition.

Metaloproteinases da Matriz e seus Inibidores Teciduais na Amiloidose Cardíaca: Relação com Alterações Miocárdicas Estruturais e Funcionais e com Deposição de Substância Amilóide de Cadeias Leves.

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Andreia Biolo, Sujata Ramamurthy, Lawreen H. Connors, Carl J. O'Hara, Hans K. Meier-Ewert, Pamela T. Soo Hoo, Douglas B. Sawyer, David S. Seldin and Flora Sam

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Matrix Metalloproteinases and Their Tissue Inhibitors in Cardiac Amyloidosis

Relationship to Structural, Functional Myocardial Changes and to Light Chain Amyloid Deposition

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Background—Cardiac amyloidosis is characterized by amyloid infiltration resulting in extracellular matrix disruption. Amyloid cardiomyopathy due to immunoglobulin light chain protein (AL-CMP) deposition has an accelerated clinical course and a worse prognosis compared with non–light chain cardiac amyloidoses (ie, forms associated with wild-type or mutated transthyretin [TTR]). We therefore tested the hypothesis that determinants of proteolytic activity of the extracellular matrix, the matrix metalloproteinases (MMPs), and their tissue inhibitors (TIMPs) would have distinct patterns and contribute to the pathogenesis of AL-CMP versus TTR-related amyloidosis.

Methods and Results—We studied 40 patients with systemic amyloidosis: 10 AL-CMP patients, 20 patients with TTR-associated forms of cardiac amyloidosis, ie, senile systemic amyloidois (involving wild-type TTR) or mutant TTR, and 10 patients with AL amyloidosis without cardiac involvement. Serum MMP-2 and -9, TIMP-1, -2, and -4, brain natriuretic peptide values, and echocardiography were determined. AL-CMP and TTR-related amyloidosis groups had similar degrees of increased left ventricular wall thickness. However, brain natriuretic peptide, MMP-9, and TIMP-1 levels were distinctly elevated accompanied by marked diastolic dysfunction in the AL-CMP group versus no or minimal increases in the TTR-related amyloidosis group. Brain natriuretic peptide, MMPs, and TIMPs were not correlated with the degree of left ventricular wall thickness but were correlated to each other and to measures of diastolic dysfunction. Immunostaining of human endomyocardial biopsies showed diffuse expression of MMP-9 and TIMP-1 in AL-CMP and limited expression in TTR-related amyloidosis hearts.

Conclusions—Despite comparable left ventricular wall thickness with TTR-related cardiac amyloidosis, AL-CMP patients have higher brain natriuretic peptide, MMPs, and TIMPs, which correlated with diastolic dysfunction. These findings suggest a relationship between light chains and extracellular matrix proteolytic activation that may play an important role in the functional and clinical manifestations of AL-CMP, distinct from the other non–light chain cardiac amyloidoses. (***Circ Heart Fail.*** **2008;1:249-257.)**

Key Words: amyloid ■ cardiomyopathy ■ metalloproteinases ■ remodeling ■ immunoglobulin light chains

Cardiac amyloidosis, a rare disorder, is characterized by amyloid fibril deposition in the heart, resulting in a restrictive cardiomyopathy that manifests late with heart failure (HF) and conduction abnormalities.[1–3] Amyloid infiltration leads to extracellular matrix (ECM) disruption resulting in diastolic dysfunction from progressive thickening and stiffening of the myocardium.[1,4] There are several types of cardiac amyloidoses, which are classified according to the biochemical nature of the amyloid deposit. In "primary" or immunoglobulin light chain amyloidosis (AL), fibrils are formed from aggregated subunits (or fragments thereof) of an amyloidogenic monoclonal light chain protein.[5] Transthyretin (TTR), normally a plasma-circulating protein, can also form amyloid deposits in myocardial tissue. Wild-type TTR is responsible for the cardiomyopathy in age-related senile systemic amyloidosis (SSA). Heritable mutations in TTR (ATTR) can result in cardiac or neuronal deposition of amyloid protein.[3,6] Of these disease types, AL-cardiac amy-

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loidosis (AL-CMP) has the worse prognosis, an aspect that is seemingly disproportionate to the structural involvement of the amyloid fibril infiltration in the myocardium.[7] A partial explanation may lie in studies, which demonstrate that light chains derived from patients with AL-CMP have direct effects on cardiomyocyte function, exerting negative inotropic effects and impairing excitation contraction coupling via increased oxidant stress.[8,9]

An alternative/complementary explanation that has not to our knowledge been explored is whether AL versus TTR amyloid fibrils differentially alters ECM turnover, a critical process in the heart for proper maintenance of myocyte–myocyte force coupling and proper myocardial function. Therefore, extracellular amyloid fibrils[4] have a high likelihood of disrupting the matrix homeostasis. Matrix homeostasis and composition are determined, in part, by collagen degradation, which are under the control of matrix metalloproteinases (MMPs), a family of zinc-dependent interstitial enzymes, and their tissue inhibitors (TIMPs). In nonamyloid cardiomyopathy, circulating MMPs and TIMPs are associated with progressive myocardial remodeling and dysfunction.[10–13] We hypothesized that light chain amyloid deposition in the heart would alter the ECM homeostasis, activate the degradation system, and thus contribute to the pathogenesis of AL-CMP, whereas other forms of cardiac amyloid would result in less ECM activation. We tested this hypothesis indirectly by measuring circulating levels of selected MMPs and TIMPs in 3 patient groups: (1) AL-CMP, (2) cardiac amyloidosis due to wild-type TTR (SSA) or mutant TTR (ATTR), and (3) light chain amyloidosis featuring renal disease without cardiac involvement (AL-renal).

Methods

Patient Data Collection

Forty age-matched patients for whom echocardiographic data and serum samples were available were selected for study, based on amyloid type. Clinical and laboratory evaluations were performed in the Amyloid Treatment and Research Program at Boston Medical Center, between November 2003 and April 2007. All subjects consented to participate in a research study under a protocol approved by the Boston University Medical Center Institutional Review Board. Subjects had biopsy-proven amyloidosis confirmed by positive Congo red staining of tissue specimens. Subjects underwent a medical history and physical examination, routine laboratory tests (eg, electrolytes, brain natriuretic peptide [BNP], blood count), 24-hour urine collection, 12-lead electrocardiography, chest x-ray examination, and echocardiography with Doppler study. AL amyloidosis is associated with a plasma cell dyscrasia and typical findings include the production of a clonal immunoglobulin light chain in the bone marrow with the presence of light chain in the serum and/or urine. Therefore, all patients were evaluated for a plasma cell dyscrasia by serum and urine immunofixation electrophoresis and by bone marrow biopsy with immunohistochemical examination to determine the presence of a monoclonal population of plasma cells.

Amyloid cardiac involvement was determined by a history of HF with myocardial wall thickening on echocardiogram (without a history of hypertension or valvular disease), low voltage on surface ECG or by an endomyocardial biopsy specimen that demonstrated amyloid deposits. Clinical HF was determined by history and physical examination followed by New York Heart Association functional classification of HF severity. AL amyloidosis was excluded if monoclonal plasma cells were absent in the bone marrow and no monoclonal gammopathy was detected in the serum or urine. Once AL amyloidosis was excluded, patients underwent screening for ATTR amyloidosis by isoelectric focusing of serum designed to detect the presence of mutant transthyretin.[14,15] Direct DNA sequencing of the TTR gene validated a positive result by isoelectric focusing and the specific mutation was identified. If both AL and ATTR were excluded, a diagnosis of SSA was made in the appropriate clinical setting.

Echocardiography

Two-dimensional and Doppler echocardiography were performed at baseline as previously described[16,17] using the Vingmed Vivid Five System (GE Vingmed, Milwaukee, Wis) with a 2.5-MHz phased-array transducer. Echocardiograms were performed and analyzed in a blinded manner. Measurements of systolic and diastolic chamber dimensions and wall thickness were obtained from 2D imaging according to the recommendations of the American Society of Echocardiography.[18] Left ventricular wall thickness (LVWT) is derived from an average of the interventricular septum and posterior wall thickness. Left ventricular (LV) mass was derived from the formula described by Devereux et al[19] 2D echocardiographic data were analyzed for LV size and function and myocardial characteristics. Adequate Doppler tracings were available for all patients. Left ventricular end-diastolic and end-systolic volumes were calculated from 2D echocardiographic dimensions as previously validated[20]: end-diastolic volume=4.5 (LV diastolic dimension)^2 and end-systolic volume=3.72 (LV systolic dimension)^2. These measurements are reliable only in subjects without a regional wall motion, ie, only validated in symmetrically contracting ventricles with normal ejection fraction.[20] From these volumes, stroke volume was estimated as end-diastolic volume−end-systolic volume. Cardiac output was calculated as stroke volume×heart rate. Similarly, relative wall thickness was calculated as (2×posterior wall thickness)/left ventricle end-diastolic diameter. Transmitral Doppler LV filling recordings were performed from the apical 4-chamber view and analyzed for diastolic filling indexes, including peak E- and A-wave velocities and their ratio. Tissue Doppler imaging was used to determine the myocardial velocity of the mitral annulus to derive e-prime (e′).

Biomarker Analysis

Blood samples were obtained at the first visit to the Amyloid Treatment and Research Program, before initiation of treatment. BNP values were measured, using the ADVIA Centaur assay (Siemans Healthcare Diagnostics), immediately after blood collection as part of routine laboratory testing. Serum samples were kept at −80°C for other assays. Gelatinases (MMP-2 and MMP-9) and tissue inhibitors of MMPs (TIMP-1, TIMP-2) were measured with commercially available ELISA kits from Amersham Pharmacia Biotech, (Buckinghamshire, United Kingdom) and the TIMP-4 kit from R&D Systems (Minneapolis, Minn).

Endomyocardial Biopsies

Myocardial tissue samples were obtained from the right ventricular septal endomyocardium, in subjects in whom the diagnosis of cardiac amyloidosis needed to be made (6 to 8 samples for each patient). This was performed from the right internal jugular vein with the use of combined fluoroscopic and echocardiographic guidance. Samples were placed in room temperature in a fixative (10% neutral buffered formalin) with a sterile needle. Endomyocardial biopsy tissue was embedded in paraffin and serial sections obtained. Congo red staining was performed on 4 to 6 μm sections to confirm amyloidosis. The remaining slides were preserved for immunohistochemistry.

Immunohistochemistry

Paraffin sections were deparaffinized, hydrated, and blocked with hydrogen peroxide for 10 minutes. The sections were then rinsed 3 times in Tween (0.05%) Tris (0.05 mol/L) buffer solution (TTBS, DakoCytomation, Glostrup, Denmark) before applying the primary antibody. Anti-MMP-9 was diluted (1:25) in antibody diluent from Dako and anti-TIMP-1 was diluted (1:50) in antibody diluent from Dako from a stock of 500 μg/mL (R&D Systems, Minneapolis, Minn). Tissue sections and antibodies were incubated overnight at 4°C, rinsed 3 times in TTBS, and incubated in HRP-labeled polymer

Table 1. Demographics and Clinical Characteristics

	AL-Renal N=10	AL-CMP N=10	SSA-ATTR N=20
Age, yr	72±1	74±2	73±1
Gender, male	5 (50)	7 (70)	17 (85)
BSA, m^2	1.78±0.07	1.82±0.06	1.83±0.05
BMI, kg/m^2	27±1	25±1	26±1
Hemodynamics			
Heart rate, bpm	76±5	77±4	73±2
Systolic BP, mm Hg	146±9	110±4*	126±3*†
Diastolic BP, mm Hg	76±4	69±2	77±2
Monoclonal protein in serum or urine			
Serum κ/λ			
κ >50%	2 (20)	0 (0)	
λ >50%	8 (80)	10 (100)	
Kidney involvement			
Nephrotic syndrome >2.5	5 (50)	2 (20)	0
Creatinine ≥1.5	5 (50)	2 (20)	1 (5)
Serum creatinine, mg/dL	2.1±0.4	1.2±0.1*	1.1±0.1*
Clinical evidence of cardiac involvement/heart failure			
NYHA >I	. . .	10 (100)	18 (90)
IVS thickness ≥1.5 cm	0	6 (60)	17 (85)
EF <50%	0	5 (50)	9 (45)
Restrictive disease (E/A >2.5 and/or lateral e′ <5)	0	7 (70)	10 (50)

Data are presented as mean±SE or n (%). BSA indicates body surface area; BMI, body mass index; NYHA, New York Heart Association; IVS, inter-ventricular septum; EF, ejection fraction.

*$P<0.05$ versus AL-renal.

†$P<0.05$ versus AL-CMP.

(DakoCytomation, Glostrup, Denmark) for 30 minutes. They were then rinsed with TTBS, treated with diaminobenzidine for ≈5 minutes, rinsed 3 times with ddH_2O, and counterstained in Harris hematoxylin for 30 seconds. After counterstaining, sections were rinsed with H_2O for 5 minutes, dipped twice in 0.25% acid alcohol, briefly rinsed in H_2O, and placed into 1% ammonia for 10 to 20 seconds before dehydrating and mounting the slides. Sections were visualized under bright-field microscopy and images were recorded using an Optronics camera with Bioquant Image software.

Statistical Analysis

Continuous variables are described as mean±standard error or median (interquartile range). Categorical variables are described as number of patients and percentages. Differences between all 3 groups regarding echocardiography characteristics, BNP, MMPs, and TIMPs were determined using ANOVA and Turkey multiple-comparison post hoc tests, or Kruskal-Wallis test for nonnormally distributed variables. Spearman correlation test was used to evaluate the correlation between echocardiographic parameters, hemodynamics, BNP, MMPs, and TIMPs. All analyses were conducted using SPSS software, version 11.5 (SPSS Inc, Chicago, Ill).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient Characteristics

Forty patients were included in the study: 10 AL-CMP patients, 20 patients with cardiac amyloidosis due to wild-type TTR (SSA) or ATTR, and 10 patients with AL amyloidosis featuring renal disease without cardiac involvement (AL-renal). One hundred percent of patients were white in AL-CMP, 75% in SSA-ATTR, and 80% in AL-renal. Additional clinical characteristics for each group are shown in Table 1. Age, body surface area, and body mass index were similar among all groups. Most of the patients were male. Systolic blood pressure was lowest in the AL-CMP group, but within the normal range. Serum free light chain measurements, revealed a predominance of λ over κ in AL-CMP and AL-renal. In the SSA-ATTR group, patients had a normal serum and urine profile. Renal function was most impaired in the AL-renal group. Almost all patients with cardiac amyloidosis (AL-CMP and SSA-ATTR) had clinical HF symptoms. Many of these patients had severe cardiac thickening and evidence of systolic and diastolic dysfunction.

LV Size, Structure, and Function

The echocardiographic data for all groups is shown in Table 2. The LV mass was significantly higher in the AL-CMP and SSA-TTR groups when compared with AL-renal group, which had no pathological LVWT. Both AL-CMP and SSA-ATTR groups had marked and comparable LVWT and concentric hypertrophy. The average calculated end-systolic volume was slightly increased in the SSA-TTR group, demonstrating that non–light chain deposition in the heart is associated with slight LV dilation.

Table 2. Echocardiography Data

	AL-Renal N=10	AL-CMP N=10	SSA-ATTR N=20
LV myocardial characteristics			
LV mass, g	144±13	226±24*	281±20*
LV mass index, g/m^2	80±5	123±11*	152±9*
Interventricular septum, cm	1.08±0.05	1.51±0.08*	1.62±0.04*
Posterior wall, cm	0.99±0.03	1.52±0.09*	1.51±0.06*
RWT	0.48±0.02	0.82±0.06*	0.72±0.04*
EDV/LV mass, mL/g	0.56±0.03	0.31±0.03*	0.31±0.02*
LV size			
Left atrium, cm	3.5±0.2	4.0±0.1*	4.4±0.1*†
LV EDD, cm	4.2±0.2	3.8±0.2	4.3±0.2
LV ESD, cm	2.6±0.2	3.0±0.2	3.3±0.2*
EDV, mL	79±6	67±6	86±7
EDV/BSA, mL/m^2	45±4	36±3	46±3
ESV, mL	25±3	34±4	42±4*
ESV/BSA, mL/m^2	14.3±1.9	18±1.9	22.6±2.0*
LV function			
Fractional shortening, %	39±2	23±2*	24±2*
Ejection fraction, %	68±2	50±3*	52±2*
SV, mL	54±4	33±3*	43±3
SV/BSA, mL/m^2	30±2	18±1*	24±2*†
Cardiac output, L/min	4.2±0.5	2.5±0.3*	3.2±0.2*
Cardiac index, L/min/m^2	2.3±0.3	1.4±0.1*	1.7±0.1*
Mitral Doppler flow			
E velocity, cm/s	69±7	94±8*	80±8
A velocity, cm/s	95±6	69±14	49±7*
E/A ratio	0.73±0.05	1.83±0.36*	2.31±0.35*
DT, ms	291±42	226±15	242±26
IVRT, ms	90±9	97±7	89±5
Tissue Doppler			
e′ lateral, cm/s	8.2±0.7	4.2±0.6*	5.2±0.4*
LA pressure			
E/e′	9±2	25±3*	17±2*†

Data are presented as mean±SE. RWT indicates relative wall thickness; EDD, end-diastolic diameter; ESD, end-systolic diameter; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; DT, deceleration time; IVRT, isovolumic relaxation time.

*$P<0.05$ versus AL-renal.

†$P<0.05$ versus AL-CMP.

In the AL-CMP and SSA-ATTR groups, LV fractional shortening and calculated ejection factor were significantly lower and in the "mildly abnormal" range. Calculated stroke volume, cardiac output, and cardiac index were all decreased in AL-CMP versus AL-renal and SSA-ATTR, reflecting impaired hemodynamics with light chain amyloid deposition. There was evidence of diastolic dysfunction in both AL-CMP and SSA-ATTR (increased E/A ratio and lower early diastolic mitral annular motion [e′] velocity). The E/e′ ratio revealed that left atrial (LA) pressure was significantly higher in AL-CMP.

BNP and ECM Proteolytic Marker Profile

Despite a comparable degree of increased cardiac wall thickness in the AL-CMP and SSA-ATTR groups, AL-CMP patients showed a marked increase in BNP values (2318±773 ng/mL), compared with a modest increase observed in the SSA-ATTR group (360±53 ng/mL; $P<0.01$; Figure 1A). Likewise, TIMP-1 values were increased in AL-CMP (1161±90 ng/mL) versus AL-renal (876±53 ng/mL; $P=0.01$) and SSA-ATTR (912±67 ng/mL; $P<0.05$; Figure 1B). In contrast, MMP-9 levels were significantly increased in both AL groups (23±6 ng/mL for AL-CMP, 18±3 ng/mL for AL-renal) versus the SSA-ATTR group (10±1 ng/mL; $P<0.05$ versus AL-CMP and Al-renal; Figure 1C). The other markers, TIMP-2, TIMP-4 and MMP-2, were not significantly altered in any of the 3 groups (Figures 1D through 1F).

Despite a similar clinical course and the lack of light chains in SSA and ATTR cardiac amyloidoses, there is evidence that the mechanism for amyloid deposition in SSA and ATTR may differ.[21] We compared whether the ECM proteolytic system and BNP levels differed between SSA and ATTR groups. BNP, TIMP-1, TIMP-2, TIMP-4, and MMP-2 were similar between the 2 groups, whereas MMP-9 levels were higher in the SSA (13.7±6.2 ng/mL) versus the ATTR group (7.2±4.1 ng/mL; $P=0.02$). Both set of values were significantly lower than those in the AL-CMP group (23±6 ng/mL).

Correlations Between Measures of the ECM Proteolytic System, BNP, and Echocardiographically Derived Measures of Cardiac Remodeling

We further evaluated whether we could correlate the degree of LVWT and LV mass and functional impairment to serum concentrations of BNP and markers of the ECM proteolytic system in patients with cardiac amyloid disease (n=30). There were no significant correlations between LV mass or LVWT to BNP, MMPs, and TIMPs. Because BNP is increased in cardiac amyloidosis and may reflect cardiomyocyte damage/toxicity, we further evaluated if BNP levels were correlated to ECM proteolytic activation. As shown in Figures 2A through 2D, there was a positive correlation between BNP levels and MMP-9, TIMP-1, MMP-2 levels and the ratio of MMP-9/TIMP-1.

Diastolic function was impaired in both AL-CMP and SSA-ATTR (Table 2). Interestingly, we found a significant negative correlation with e′ lateral tissue Doppler and BNP, TIMP-1, and MMP-9 (Figures 3A through 3C).

Myocardial Biopsy MMP-9 and TIMP-1 Analyses

The presence and abundance of myocardial MMP-9 and TIMP-1 expression from patients with cardiac amyloidosis were investigated in endomyocardial biopsies (Figures 4A through 4C). MMP-9 expression was diffusely increased in AL-CMP (n=5) cardiomyocytes versus sparse expression in non–light chain cardiac amloidosis (SSA; n=4). In AL-CMP, MMP-9 was visibly absent from the ECM (intersitium), which has been replaced by light chain amyloid deposits. In addition, there was scant MMP-9 expression in the perivascular areas. Likewise, TIMP-1 expression (Figure 4C) was

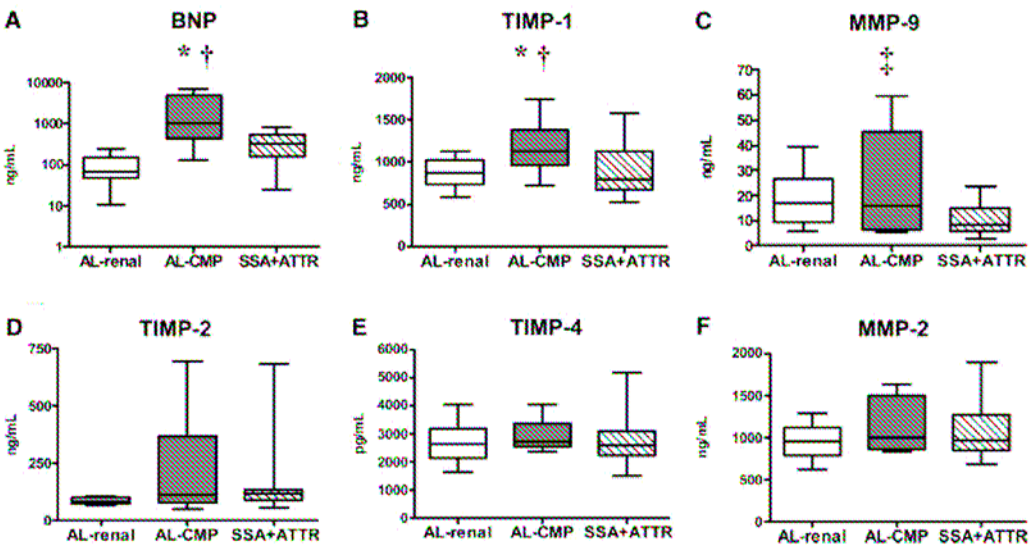

Figure 1. BNP and the ECM proteolytic marker profile in cardiac and noncardiac amyloidosis. A, BNP; B, TIMP-1; C, MMP-9; D, TIMP-2; E, TIMP-4; F, MMP-2. *P=0.01 versus AL-renal; †P<0.01 versus SSA-ATTR; **P<0.05 versus AL-renal; ‡P<0.05 versus SSA-ATTR.

increased in cardiomyocytes and also distributed diffusely throughout the myocardium in AL-CMP versus SSA hearts.

Discussion

This is the first study to evaluate the ECM proteolytic system in different forms of cardiac amyloidosis. Both light chain (AL-CMP) and non–light chain (SSA-ATTR) cardiac amyloidosis resulted in an increased but comparable LVWT and an increase in LV mass. Despite this structural similarity, the presence of cardiotropic amyloidogenic light chains resulted in distinct increases in serum concentrations of BNP, MMP-9, and TIMP-1, which correlated with measures of diastolic dysfunction in AL-CMP. Similarly, increased MMP-9 and TIMP-1 were present in myocardial tissue containing light chain amyloid deposits. These findings suggest a relationship between light chain-amyloid disease and ECM proteolytic activation, which might play a role in the progression of cardiac amyloid disease and the distinct differences in prognosis between these groups of patients.

BNP Levels

In cardiac amyloidosis, BNP is released from cardiomyocytes and there is direct evidence of an increase in BNP gene and protein expression in ventricular myocytes.[22] The inactive proform of BNP, NT-proBNP, is elevated in AL-CMP[23–25] and appears to precede overt cardiac involvement.[23] Furthermore, BNP values have been shown to correlate with prognosis[25] and response to therapy.[23–25] In patients with cardiac

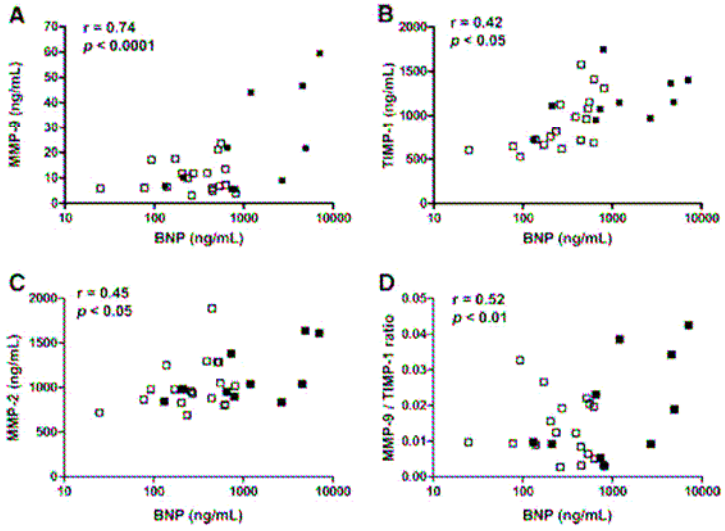

Figure 2. The relationship between BNP and MMP-9 (A), TIMP-1 (B), MMP-2 (C), and MMP-9/TIMP-1 (D). Closed squares indicate AL-CMP; open squares, SSA-ATTR group.

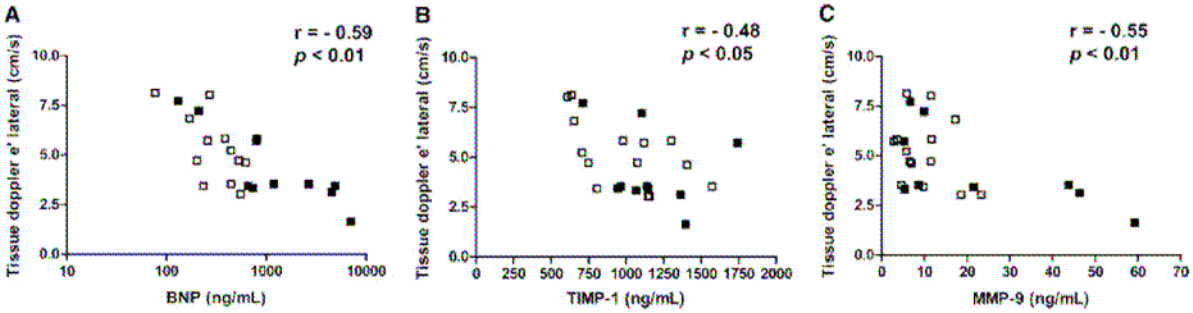

Figure 3. The relationship between tissue Doppler e' and BNP (A), TIMP-1 (B), and MMP-9 (C). Closed squares indicate AL-CMP; open squares, SSA-ATTR group.

disease and no amyloid, a good correlation exists between BNP and LVWT, diastolic dysfunction and end-diastolic wall stress.[26–28] In our study, an interesting finding was that the highest BNP levels were present in the AL-CMP group, despite the fact that the SSA-ATTR group had similar cardiac mass, LVWT and severe diastolic dysfunction. Taken together, these findings suggest that increased BNP in cardiac amyloidosis may reflect not only elevated LV filling pressure,[22,29] but also the direct cardiac myocyte damage due to extracellular deposition of light chains in AL-CMP.[8,9,29]

There appears to be a paradoxical relationship between LA size and E/e' in AL-CMP versus SSA-ATTR groups. The greater LA size but lower E/e' in SSA-ATTR could be explained by a longer duration of cardiac disease, because LA enlargement is a marker of severity and the chronicity of diastolic dysfunction and the magnitude of LA pressure

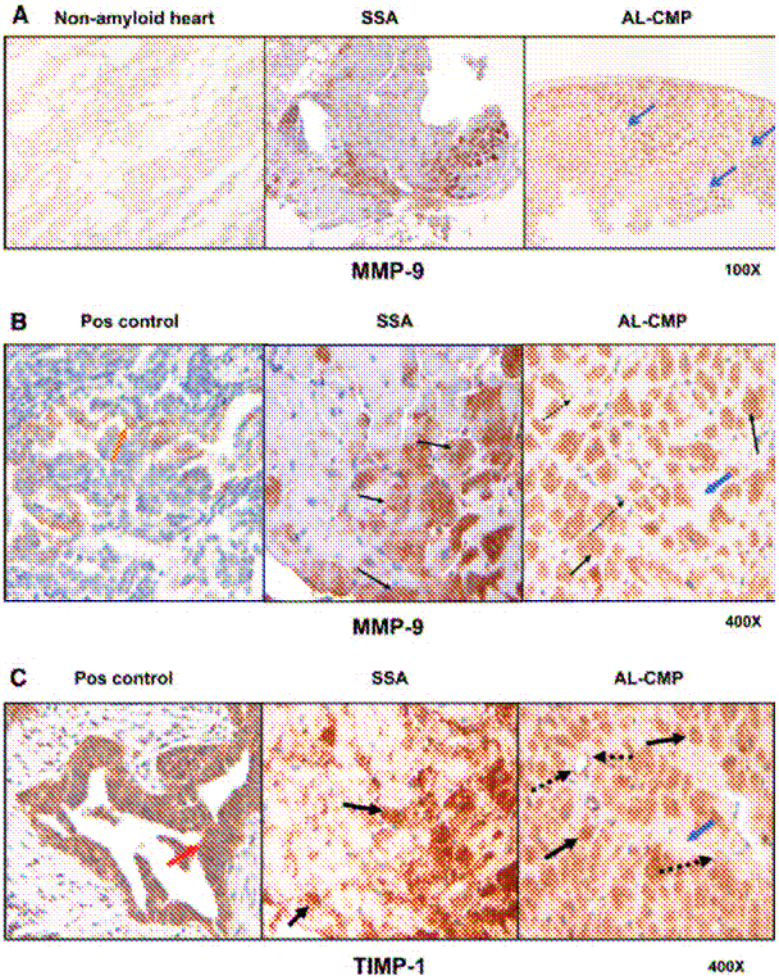

Figure 4. Immunohistochemical analyses of MMP-9 and TIMP-1 in amyloidotic endomyocardial biopsy. A, There is no MMP-9 staining in the normal, nonamyloid heart. MMP-9 expression is more diffuse in AL-CMP than SSA heart biopsies. Solid blue arrows indicate amorphous amyloid deposition in the ECM. (Magnification ×100.) B, MMP-9 expression in AL-CMP and SSA heart biopsies. Solid black arrows indicate staining in cardiomyocytes; dashed back arrows, MMP-9 staining in the interstitium; red arrows, MMP-9 expression in (+) control; blue arrows, amyloid deposits in the ECM. (Magnification ×400.) C, TIMP-1 expression is more diffuse in AL-CMP than SSA heart biopsies. Solid arrows indicate TIMP-1 staining in cardiomyocytes; dashed arrows, TIMP-1 staining in the interstitium; red arrows, TIMP-1 expression in (+) control; blue arrows, amyloid deposition in the ECM. (Magnification ×400.)

elevation.[30–32] SSA-ATTR presents in elderly people and has a more insidious course resulting in slowly progressive LA dilatation and by the time they present with HF may have a lower LA pressures (E/e').

MMPs and TIMPs

Similar to cysteine proteinases that degrade the ECM in local areas,[33] MMPs and TIMPs may determine the rate and extent of matrix turnover (ie, collagen degradation) in the heart. In nonamyloid cardiac remodeling, a relationship has been suggested between wall stress and MMP expression in an experimental model of myocardial infarction.[34] MMP expression was associated with increased LV end-systolic wall stress[34] and MMP inhibition ameliorated adverse structural, cardiac remodeling.[35] Similarly both MMP-2 and MMP-9 may play a role in cardiac hypertrophy and remodeling.[36–38]

Distinct patterns of MMP and TIMP expression occur in the LV myocardium of patients with systolic HF and diastolic HF. In patients with diastolic HF from hypertension[12] or aortic stenosis,[39] there is a decreased matrix degradation because of MMP downregulation and TIMP upregulation. In systolic HF, such as in dilated cardiomyopathy, there is increased matrix degradation because of MMP upregulation.[40] In aortic stenosis, when the LVEF eventually declines, a shift occurs between proteolysis and antiproteolysis.[41] In our study, AL-CMP is associated with increased circulating and myocardial MMP-9 and its inhibitor TIMP-1. Circulating MMP-9 levels are increased in both cardiac and noncardiac forms of AL amyloidosis. However, circulating TIMP-1 levels are increased in only the cardiac form (AL-CMP). This would indicate that perhaps matrix degradation is impaired in the heart and consistent with the above relationship of MMP and TIMP seen in diastolic HF. Interestingly there is no difference in fibrosis from endomyocardial biopsies in cardiac amyloidoses (data not shown). Noticeably, other endogenous modulators are responsible for effective matrix destruction, such as cathepsins,[33] which were not studied here and may play an important role.

Our study suggests that AL-amyloid, and thus light chain cardiac amyloid deposition, is associated with altered markers of matrix turnover (increased circulating MMP-9 and TIMP-1 levels) and increased expression in the myocardium. In AL amyloidosis, the presence of TIMP-1 and TIMP-2 has been observed in pathological sections of liver, kidney, and spleen.[42] It has been proposed that altered expression of MMPs and TIMPs play a role in mesangial remodeling in renal amloidosis.[43] Light chain deposition alters the cellular redox state in isolated cardiomyocytes, resulting in impairment of cardiomyocyte contractile function and calcium handling.[8,9] Reactive oxygen species alter myocardial MMP activity through translational and posttranslational mechanisms, activating MMPs and decreasing fibrillar collagen synthesis in cardiac cells. Taken together, these data suggest that activation of the ECM proteolytic system observed in AL-CMP might result, at least in part, from light chain toxicity, and contribute to accelerated clinical phenotype and disease progression observed in light chain cardiac amyloidosis.

The present study has several limitations. First, because this is a cross-sectional analysis, it does not allow conclusions regarding cause and effect. Further insight into the role of MMPs and TIMPs in depositional cardiomyopathies might be gained by intervention studies and longitudinal follow-up of patients with cardiac amyloidosis. Second, the sample size is relatively small and it is possible that relationships between BNP and other biomarkers (MMP-2, TIMP-1 and TIMP-4) would be observed in a larger group of subjects. However, our significant findings in this small sample size of patients may serve to highlight the role of these markers. In addition, other MMP and TIMP species are expressed within the human myocardium but only MMP-2, -9, TIMP-1, -2, and -4 levels were examined in the current study. Third, although AL amyloidosis is a systemic disease and we measured circulating biomarker levels, renal dysfunction could impact those levels, resulting in false-positive findings. However, there was no correlation between renal dysfunction, defined as the presence/absence of renal involvement or as creatinine levels, to BNP levels or ECM proteolytic markers, except for MMP-9, which was increased in both AL groups (data not shown). Moreover, a direct assessment of tissue MMP activity (ie, zymography) was not performed. Given our positive findings, further studies are required to identify other MMPs and TIMPs that may be altered in AL-CMP patients. Finally, differences in medication that can affect fibrosis across the 3 groups may be a potential limitation. However, most patients with cardiac amyloidosis were on minimal cardiac medications, and there were no differences in the distribution of the types of medications between the 3 groups of patients.

In conclusion, our study demonstrates that light chain cardiac amyloidosis, despite its structural similarities to other forms of cardiac amyloidosis (SSA or ATTR), results in disproportionate and markedly increased levels of BNP and ECM proteolytic markers, as well as impaired hemodynamics and diastolic dysfunction. These findings may provide insight into the mechanisms for accelerated amyloid disease progression and impaired prognosis associated with light chain cardiac involvement. Future studies will determine whether interventions aimed to interrupt this process may benefit this group of patients.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Cardiac amyloidosis is characterized by amyloid fibril deposition in the heart with extracellular matrix disruption and progressive wall thickening and stiffening, resulting in a restrictive cardiomyopathy. Systemic amyloidosis featuring cardiac involvement (AL-CMP) is caused by immunoglobulin light chain protein deposition in the heart that may manifest with congestive heart failure, arrhythmias, and death within 6 months, if untreated. Although cardiac amyloidosis may be related to other non–light chain proteins (ie, amyloidosis associated with either wild-type transthyretin or a mutant transthyretin), the prognosis for patients with AL-CMP is worse and the mechanism poorly understood. Direct cardiotoxicity from light chains has been implicated. The current study shows that despite similar structural myocardial involvement (wall thickening and diastolic dysfunction) for both AL amyloidosis and the transthyretin-related forms, AL-CMP patients had higher brain natriuretic petide levels and increased markers of extracellular matrix proteolytic activity (MMP-9 and TIMP-1), which were not associated with the degree of wall thickening. Therefore, structural abnormalities by echocardiography may not reflect the severity of AL-CMP. The presence of light chains in AL-CMP, as well as increased levels of brain natriuretic petide and markers of extracellular matrix proteolytic activity, may reflect additional mechanisms that contribute to the accelerated clinical disease that has been reported. Our findings represent an initial step to increasing our understanding of the pathophysiology of this poorly understood disease. Subsequent steps would focus on the association of these markers with clinical course and prognosis and whether interventions directed to interrupt these processes would prevent disease progression and improve clinical outcome.

Conclusões

I. Episódios de ICAD estão associados com aumentos transitórios em marcadores de lesão de cadiomiócitos e de ativação da matriz extracelular, podendo desta forma resultar em remodelamento patológico acelerado e contribuir para desfechos a longo prazo.

II. A rigidez arterial está aumentada na ICAD e contribui para o aumento da pós-carga arterial. O tratamento efetivo da descompensação resulta em redução da rigidez arterial e aumento da eficiência ventricular, sugerindo que alterações na rigidez arterial contribuam para a fisiopatologia da ICAD.

III. Pacientes com amiloidose cardíaca primária, associada a imunoglobulinas de cadeias leves, têm atividade proteolítica aumentada da matriz extracelular quando comparados às outras formas de amiloidose cardíaca (senil e mutação da transtirretina), apesar de comparável comprometimento cardíaco estrutural. A toxicidade das cadeias leves sobre a matriz extracelular pode contribuir para a progressão clínica acelerada da amiloidose primária.