Seroreactivity Against Recombinant Citrullinated Myosin Is Associated with Measures of Diastolic Dysfunction in Patients with Rheumatoid Arthritis

Christian Geier[1], Justyna Fert-Bober[2], Jon T Giles[1], Cesare Russo[1], Aylin Tugcu[1], Sabahat Bokhari[1], Jenny Van Eyk[2], Joan Bathon[1]

[1]Columbia University, New York, NY and [2]Cedars-Sinai Hospital, Los Angeles, CA

COLUMBIA

COLUMBIA UNIVERSITY IRVING MEDICAL CENTER

Abstract

Background/Purpose:
Diastolic dysfunction and heart failure with preserved EF are more prevalent in RA. We have previously shown increased staining for citrullinated substrates in necropsied hearts of RA patients. We hypothesized that individuals with RA may generate antibodies against citrullinated myocardial proteins and that such antibodies may be associated with left ventricular (LV) dysfunction.

Methods:
59 sera from RA patients enrolled in a cohort study were incubated with uncitrullinated or citrullinated (exposed to peptidyl-arginine-deiminase-2 [PAD-2]) myocardial proteins (actin, myosin, tropomyosin, and troponin). Fluorescent anti-human IgG Fc antibody was added, rinsed, and mean fluorescence intensities (MFI) were recorded. Demographics, RA characteristics, and measures of LV function were compared between highest and lowest MFI tertiles for each protein in both citrullinated and uncitrullinated forms. The associations of anti-myocardial antibodies with measures of cardiac function, assessed by 3D echocardiography, were modeled using generalized linear models, adjusting for relevant confounders (variables associated with both LV function and seroreactivity to anti-myocardial proteins).

Results:
Patient sera with the highest tertile of seroreactivity against citrullinated (but not uncitrullinated) myosin showed multiple differences ($p < 0.05$) in measures of diastolic function: E/A ratio (0.95 vs 1.05), mean S wave (8.80 vs 10.09) and E/E' ratio (9.64 vs 7.86) all indicated better diastolic function for patients in the lowest tertile. Systolic function between the seroreactivity tertiles was not different. Levels of other myocardial protein antibodies were not associated with diastolic function with the exception of S wave for citrullinated tropomyosin ($p = 0.021$). Multivariable analyses showed that the diastolic parameters E/E' ratio and S wave (mean) remained significantly associated after controlling for RA duration and Tender Joint Count, previously identified potential confounders (Fig. 1).

Conclusion:
These data suggest that RA patients may generate antibodies against citrullinated myosin and that this may contribute to myocardial dysfunction in RA. Analyses of additional patient sera and ultimately verifying this observation in human myocardial tissue are needed.

Introduction

- Diastolic dysfunction and heart failure with preserved EF are more prevalent in RA.[1]
- We have previously shown increased staining for citrullinated substrates in necropsied hearts of RA patients.[2]
- **We hypothesized that individuals with RA may generate antibodies against citrullinated myocardial proteins and that these antibodies are associated with left ventricular (LV) dysfunction.**

1. *Doran MF, Pond GR, Crowson CS, O'Fallon WM, Gabriel SE. Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. Arthritis & Rheumatism. 2002 Mar 1;46(3):625–31.*
2. *Giles JT, Fert-Bober J, Park JK, Bingham CO, Andrade F, Fox-Talbot K, et al. Myocardial citrullination in rheumatoid arthritis: a correlative histopathologic study. Arthritis Research & Therapy. 2012 Feb 24;14:R39.*

Results

1) RA sera were significantly more reactive to citrullinated [PAD-treated] **myocardial proteins** (Actin, Myosin, Tropomyosin and Troponin) **than to their untreated homologs** (Figure 1).

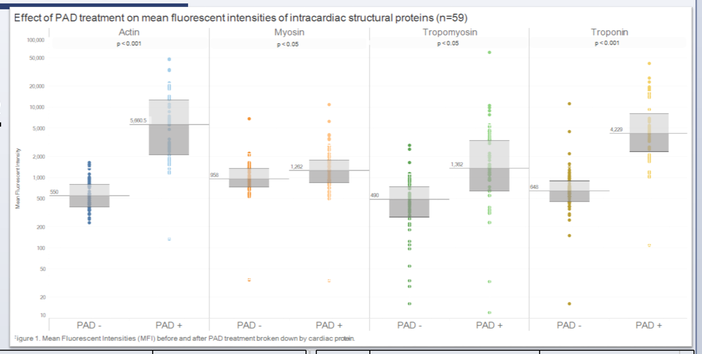


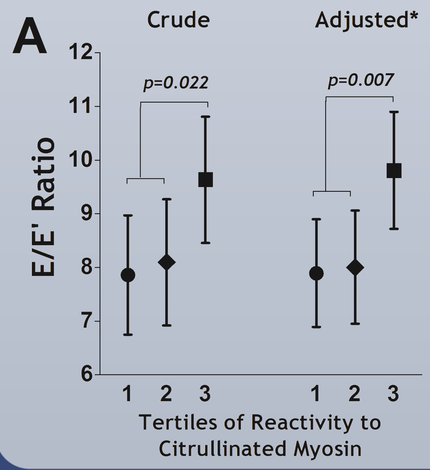
Figure 1. Mean Fluorescent Intensities (MFI) before and after PAD treatment broken down by cardiac protein.

	Uncitrullinated Myosin			*Citrullinated* Myosin		
	Lowest Tertile *n=20*	Highest Tertile *n=20*	p value	Lowest Tertile *n=21*	Highest Tertile *n=20*	p value
Demographics						
Age in years	54.05	56.70	0.49	55.05	58.95	0.32
Current smoker	2	2	1.00	2	0	0.49
Male Gender	5	0	0.05	5	3	0.70
RA characteristics						
DAS-28 (SD)	3.97 (1.13)	4.07 (1.11)	0.78	3.76 (1.14)	3.77 (1.43)	0.98
CCP in units, median [IQR]	94.50 [16.00, 250.00]	250.00 [64.00, 250.00]	0.27	83.00 [16.00, 250.00]	250.00 [250.00, 250.00]	**<0.01**
Systolic function						
EF in percent, mean (SD)	60.95 (5.88)	61.58 (5.16)	0.73	61.00 (5.20)	61.75 (5.11)	0.65
GLS (SD)	-17.11 (2.18)	-16.98 (1.76)	0.84	-17.09 (1.86)	-16.27 (1.93)	0.18
Diastolic Function						
E/E' (SD)	8.11 (3.02)	9.05 (2.86)	0.33	7.86 (2.67)	9.64 (2.68)	**0.048**
E/A (SD)	1.17 (0.41)	1.05 (0.33)	0.32	1.21 (0.43)	0.95 (0.27)	**0.026**

	Uncitrullinated Troponin			*Citrullinated* Troponin		
	Lowest Tertile *n=20*	Highest Tertile *n=20*	p value	Lowest Tertile *n=20*	Highest Tertile *n=20*	p value
Demographics						
Age in years	55.50	53.45	0.61	55.85	56.55	0.86
Current smoker	2	2	1.00	2	0	0.49
Male Gender	6	0	0.02	5	3	0.70
RA characteristics						
DAS-28 (SD)	3.66 (0.90)	4.30 (1.01)	**0.04**	3.61 (1.03)	3.66 (1.46)	0.91
CCP in units, median [IQR]	231.00 [21.25, 250.00]	250.00 [16.00, 250.00]	0.72	81.50 [16.00, 250.00]	250.00 [250.00, 250.00]	**< 0.001**
Systolic function						
EF in percent, mean (SD)	61.89 (6.31)	60.25 (4.22)	0.36	61.67 (4.93)	60.50 (5.25)	0.49
GLS (SD)	-16.31 (1.73)	-17.20 (2.02)	0.15	-17.41 (1.84)	-16.46 (1.98)	0.13
Diastolic Function						
E/E' (SD)	7.99 (2.33)	7.94 (1.66)	0.93	8.09 (2.87)	8.56 (2.53)	0.60
E/A (SD)	1.16 (0.45)	1.13 (0.27)	0.84	1.17 (0.40)	1.09 (0.33)	0.51

Table 1: Characteristics of patients with highest and lowest MFI groups. Proteins not shown: Actin, Troponin
DAS: Disease activity score CCP: cyclic citrullinated peptide EF: ejection fraction GLS: global longitudinal strain

2) *E/E'* and *E/A ratios* (measures of diastolic function) **differed significantly between highest and lowest tertiles of MFIs for citrullinated myosin** (Table 1). There were no statistically significant differences in measures of diastolic dysfunction between groups for ***uncitrullinated*** myosin ***or any of the other myocardial proteins*** (either citrullinated or uncitrullinated).



3) Multivariable analyses showed that the diastolic parameter *E/E' ratio* remained significantly associated after controlling for RA duration and Tender Joint Count, previously identified potential confounders (Figure 2)

Fig. 2: Comparison of E/E' ratio by tertile of reactivity to citrullinated myosin
Means and 95% CI depicted
* Adjusted for *RA duration* and *Tender Joint Count*

Methods

59 sera from RA patients enrolled in a cohort study were incubated with uncitrullinated or citrullinated (exposed to *peptidyl-arginine-deiminase-2* [PAD-2]) myocardial proteins (actin, myosin, tropomyosin, and troponin). Fluorescent anti-human IgG Fc antibody was added, rinsed, and mean fluorescence intensities (MFI) were recorded. Demographics, RA characteristics, and measures of LV function were compared between highest and lowest MFI tertiles for each protein in both citrullinated and uncitrullinated forms. The associations of anti-myocardial antibodies with measures of cardiac function, assessed by 3D echocardiography, were modeled using generalized linear models, adjusting for relevant confounders (variables associated with both LV function and seroreactivity to anti-myocardial proteins).

Conclusion

RA patients generate antibodies against citrullinated myosin.

The formation of such antibodies may contribute to diastolic dysfunction in RA.

Studies to elucidate the pathophysiologic mechanisms including analyses of additional patient sera and ultimately verification of this observation in human myocardial tissue are needed.

Scan QR code to download abstract