Chapter 4

Presence of anti-citrullinated protein antibodies in sera of non-rheumatic cardiovascular patients is associated with long-term mortality.

Manuscript submitted

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Abstract:

Objective:
Cardiovascular (CV) mortality is higher in patients with rheumatoid arthritis (RA), in particular when anti-citrullinated protein antibodies (ACPA) are present. Recently, ACPA have also been described in CV patients without RA.
In this study, we aimed to confirm the presence of ACPA in the serum of non-rheumatic CV patients. In addition, we aimed to assess the relation between ACPA with clinical parameters, plaque phenotype and long-term mortality.
Methods
Sera of CV patients who were included into either the AtheroExpress, Circulating Cells or MISSION! study were analyzed for presence of ACPA. After exclusion of patients with known rheumatic diseases, we analyzed the associations between ACPA positivity and clinical characteristics in all three cohorts. Furthermore, we aimed to associate ACPA with either vulnerable plaque characteristics in the AtheroExpress cohort or long-term mortality in the MISSION! study.
Results
Compared to sera from healthy controls, we detected a significantly higher percentage of ACPA positivity in the sera of patients in all three study cohorts. Clinical analysis of these ACPA negative and positive CV patients showed that presence of ACPA did not associate with general characteristics such as lipid profile, BMI or other known risk factors. Plaque phenotype did not differ between ACPA negative and positive CV patients in the AtheroExpress study. However, we observed an increased cumulative cardiac mortality in ACPA positive CV patients compared to ACPA negative CV patients in the MISSION! cohort. Corrected for age, ACPA positivity was independently associated with long-term mortality.
Conclusion
This study confirms that ACPA is present in a subpopulation of non-rheumatic CV patients. Clinical analysis showed that long-term mortality in STEMI patients without RA was independently associated with the presence of ACPA. Therefore, ACPA in patients without RA might act as a cardiovascular factor.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of the synovial tissue, which affects around 1% of the world population. The persistent synovitis leads to the breakdown of cartilage and bone that ultimately results in joint disability [1]. The generation of autoantibodies is a key characteristic of the majority of RA patients and especially anti-citrullinated protein antibodies (ACPA) are frequently found in the sera and synovial fluid of RA patients [2]. Citrullination or peptidylarginine deimination is a physiological process catalyzed by a family of enzymes called peptidyl arginine deiminases (PAD-1-4) [3]. These enzymes convert the positively charged amino acid arginine to an uncharged amino acid citrulline in the presence of relatively high calcium concentrations [3]. Clinically, ACPA is an important marker for a more progressive RA compared to ACPA$^-$ negative RA patients [4]. Additionally, ACPA positivity in RA patients is associated with a higher cardiovascular mortality [5].

The major underlying pathology of many acute cardiovascular diseases is the process of atherosclerosis. Atherosclerosis is the sub-endothelial accumulation of lipids and inflammatory cells in the arterial wall, resulting in the development an atherosclerotic plaque, generally composed of a lipid core covered by a fibrous cap. Thrombosis after rupture or erosion of such a fibrous cap can lead to occlusive vascular diseases such as myocardial infarction or stroke [6]. Citrullination of proteins is not restricted to the synovial tissue and is reported to also occur in other tissues, for example within the myocardium and inside the atherosclerotic lesion [7, 8]. In addition, PAD enzymes have been detected in high levels in atherosclerotic lesions of non-RA patients with cardiovascular disease [8]. Low levels of circulating ACPA have been reported in a small proportion of patients with acute cardiovascular syndromes in the absence of rheumatic diseases [9]. Formation of immune complexes composed of ACPA and citrullinated proteins can activate the complement cascade as well as Fc-receptor mediated activation of local leukocytes, thereby fuelling the ongoing inflammatory response. Therefore, the presence of ACPA in cardiovascular patients could potentially contribute to atherosclerotic lesion growth in the vessel wall, however up to date a causal relation between ACPA and cardiovascular diseases such as atherosclerosis has not been established.

In the current study, we aimed to determine whether ACPA is detectable in CV patients without reported RA. In addition, we aimed to associate ACPA positivity with clinical characteristics, plaque phenotype and long-term mortality in these patient cohorts.

Methods

Study population and design

Athero-Express

A total of 135 patients of the Athero-Express were included in this study. The Athero-express biobank involves patients that underwent carotid endarterectomy (CEA) in two Dutch teaching hospitals in Utrecht and Nieuwegein, the Netherlands [10]. The criteria to perform carotid endarterectomy were based on the recommendations by the Asymptomatic Carotid Atherosclerosis Study (ACAS study) for asymptomatic patients and the North American Symptomatic Carotid Endarterectomy Trial and the European Carotid Surgery Trial (NASCET study) for symptomatic patients [11–15]. The local medical ethical boards of both participating hospitals approved this study. The participating patients signed a written informed consent prior to inclusion. The patient's baseline characteristics and medical history were obtained via questionnaires and the patient medical records.

Circulating Cells

The study cohort consists of a total of 443 patients, these patients form a subgroup of the Centre for Translational Molecular Medicine (CTMM) – Circulating Cells study cohort [16]. In brief, Circulating Cells is a multi-centre study in which CAD patients scheduled for coronary angiography were included. Exclusion criteria were age <18 years, inability to give informed consent, suspected drug or alcohol abuse, serious concomitant disease and serious recent infectious disease in the last 6 weeks or suspected elevated state of the immune system, and non-cooperativeness. The Circulating Cells protocol was approved by the review board or ethics committee of each participating centre. Nine months after inclusion, patients were contacted and any major adverse cardiovascular events (MACE) that occurred in the preceding months were recorded. MACE is the primary endpoint of this study and is defined as cardiovascular disease related death, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting and cardiovascular accident.

Mission

Data for this study were used from 300 patients with ST-segment elevation myocardial infarction (STEMI), who were initially included in the MISSION! Intervention Trial. STEMI was defined as ongoing chest pain (>30 minutes), accompanied with ST-elevation (≥0.2 mV in ≥2 leads in V1-V3 or ≥0.1 mV in other leads) or presumed new left bundle branch block (LBBB) and a typical rise and fall of cardiac biomarkers. In case of out-of-hospital cardiac arrest, only patients with return of spontaneous circulation at the moment of arrival at the catheterization laboratory were included. Patients with prior myocardial infarction (n=11) or prior revascularization (n=6) were excluded. In addition, patients with evidence for rheumatic disease (n=8) were excluded for analysis. The MISSION! Intervention Trial was conducted from February 2004 to October 2006. Clinical and angiographic results in patients with STEMI treated with either Bare Metal Stents (BMS) or Sirolimus Eluting Stents (SES) during primary percutaneous coronary intervention (PCI) was evaluated [17].

Information on all-cause mortality was obtained from the Dutch Municipality Records registry. Cause of death was retrieved from general practitioners. Clinical follow-up data was collected during the 30 days, 3, 6 and

12 months outpatient clinic visits. Follow-up data on serious adverse events including myocardial infarction, revascularisation and stroke was obtained by telephone interviews at 2, 5 and 10 years after admission. The study protocol was approved by the institutional ethical committee. Written informed consent was obtained from all patients before enrolment in the study. In the current study, baseline blood samples derived from the patients before primary PCI were used for anti–citrullinated protein antibodies (ACPA) determination.

SYNTAX Scoring

To assess the complexity of coronary artery disease, coronary angiographic primary PCI images were used to calculate the SYNTAX score [18]. This was performed by an experienced interventional cardiologist.

Anti–citrullinated protein antibodies measurement

ACPA positivity was determined based on reactivity of sera against a third generation cyclic citrullinated peptide (CCP3) in a commercially available ELISA system (Quanta LiteTM CCP3.1 IgG/IgA Cat# 704550, INOVA Diagnostics Inc., US). According to supplier's manual, values above 20 aU/mL were considered CCP3.1 positive.

Statistical analysis

Normally distributed continuous variables were reported as mean and standard deviation, and compared with Student's t-test. Skewed distributed continuous variables were reported as median and interquartile range, and compared with Mann-Whitney U test. Categorical variables were reported as number and percentage, and compared with Pearson's chi-square test. Event-free survival was analyzed with Kaplan-Meijer estimates and compared between groups with the log-rank test. Cox regression was performed to assess the association between the ACPA positivity and primary and secondary outcome measures. All statistical tests were performed with SPSS software (Version 22.0, SPSS IBM Corp., Armonk, New York). P-values <0.05 assessed by two-sided tests were considered to be statistically significant.

Results

Presence of ACPA was determined by screening sera, obtained from CV patients included in the AtheroExpress, Circulating Cells and MISSION! cohorts, for reactivity towards a cyclic citrullinated peptide (CCP3).

Baseline characteristics

The baseline characteristics of patients included in the AtheroExpress are summarized in table 1. In brief, the cohort has a mean age of 67 and a male prevalence (71%), which reflects a relatively typical population of patients with vascular occlusive diseases. The majority of patients was symptomatic (74%) as illustrated by the incidence of amaurosis fugax, a TIA or a stroke, was hypertensive (86%) and used statins (69%).

Table 1: Baseline patient characteristics of AtheroExpress

Characteristic	**Median (±SD)**
Age, mean years (SD)	67 (9)
BMI, mean kg/m2 (SD)	27 (4)
	n (%) of patients
Male	96/135 (71%)
Smoker	55/134 (41%)
Diabetes mellitus	25/135 (19%)
Statin use	93/135 (69%)
Hypertension	116/135 (86%)
Hypersensitive	27/132 (20%)
History VI	4/135 (40%)
History MI	30/134 (22%)
Clinical presentation	
Asymptomatic	35/135 (26%)
Symptomatic	100/135 (74%)
Amaurosis fugax	22/135 (16%)
TIA	51/135 (38%)
Stroke	27/135 (20%)

The circulating cells cohort consist of a total of 443 patients with a mean age of 63 and a male prevalence of 71%. The majority of the patients were diagnosed with stable coronary artery disease (CAD) (81%), whereas the remaining patients suffered from unstable CAD (non-ST elevation myocardial infarction/unstable angina) (19%). Table 2 summarizes the baseline characteristics of this patient cohort.

Table 2: Baseline patient characteristics of Circulating Cells

Characteristic	**Median (±SD)**
Age, mean years (SD)	63 (10)
BMI, mean kg/m2 (SD)	27 (4)
Men (%)	314/443 (71%)
	Median (IQR)
SBP (mm Hg)	136 (122-145)
DBP (mm Hg)	78 (70-85)
Glucose (mmol/L)	6,55 (5,40-6,90)
HB (mmol/L)	8,70 (8,30-9,20)
LDL-C (mmol/L)	2,51 (1,87-3,01)
HDL-C (mmol/L)	1,08 (1,24-0,89)
Triglycerides (mmol/L)	1,57 (0,99-1,90)
hsCRP (ng/mL)	7919 (1685-7235)
WBC (x1000 cells/µL)	7,3 (5,9-8,5)
	n (%) of patients
Current smoker	97/443 (22%)
Diabetes Mellitus	20/443 (20%)
Hypertension	281/443 (64%)
Beta-Blocker	336/443 (76%)
Ca-antagonist	130/443 (29%)
Aspirin	369/443 (83%)
Vitamin K antagonist	47/443 (11%)
Statin	351/443 (79%)

The MISSION! cohort consists of a total of 275 patients with a mean age of 59 and a male prevalence of 76%. Baseline characteristics are summarized in table 3.

Table 3: Baseline patient characteristics of Circulating Cells

Characteristic		**n= 246**
Patient characteristics		
Age, mean (SD), y		58,9 (11,5)
Woman		65 (275)
Body mass index, mean (SD), kg/m2		26,6 (4,5)
Risk factors		
Treated hypertension		76 (28)
Diabetes		25 (9)
Treated hyperlipidemia		48 (17)
Current smoker		152 (55)
Family history of CVD		122 (44)
Clinical characteristics		
Out of hospital cardiac arrest		7 (3)
Cardiogenic shock		5 (2)
Anterior infarction		145 (53)
Infarct size, median (IQR), g/m2		9,1 (4,4 – 15,7)
Troponine max, median (IQR)		5.8 (2,4 – 10)
CK max, median (IQR)		1985 (966 – 3633)
Troponine max (log)		0,62 (0,55)
CK max (log)		3,3 (0,5)
Number of vessels diseased (>50%)	1	154 (56)
	2	107 (39)
	3	12 (4)
Complete revascularization		275 (100)
Culprit vessel	RCA	82 (30)
	LAD	150 (55)
	LCX	43 (16)
Proximal lesion		138 (50)
TIMI flow before intervention 0		189 (69)
Drug-eluting stent		135 (49)
Irreversible ischemia myoview		198 (72)
Irreversible ischemia myoview		107 (39)
Syntax score before pPCI (IQR)		14,5 (8,8 – 20,4)
Syntax score after pPCI (IQR)		8,9 (6,7 – 13,2)
CRP, median (IQR)		0,5 (0,0 – 6,3)
CRP (log)		4,6 (5,5)
CRP >3mg/L		108 (39)
Clinical endpoints		
Re-infarct		36 (13)
Cardiac death		13 (5)
Death		35 (13)
Re-infarct or death		65 (24)

CCP3-reactivity in the sera of CV patients

CCP3-positivity in the tested cohorts as well as a group of age matched healthy controls is depicted in figure 1. We observed that 1,9% (3/160) of the healthy controls, 8,1 % (11/135) of the Athero- Express patients, 5,3% (23/430) of the Circulating Cells patients and 10,0% (30/292) of the Mission! patient cohort showed reactivity for the CCP3 peptide. Statistical analysis showed that the percentages of CCP3 reactive antibodies in all the cohorts are significantly elevated compared to healthy controls.

Clinical characteristics

AtheroExpress

Next, we aimed to correlate ACPA positivity with clinical observations within the AtheroExpress database. We did not observe associations between ACPA positivity and any of the following plaque characteristics: fat deposition, collagen, smooth muscle cells, macrophages, microvessel density. In addition, clinical presentation (asymptomatic (n=35) or symptomatic (n=100) patients) was not associated with ACPA positivity. Furthermore, analysis of the symptomatic patients did not show any association with clinical presentation (data not shown).

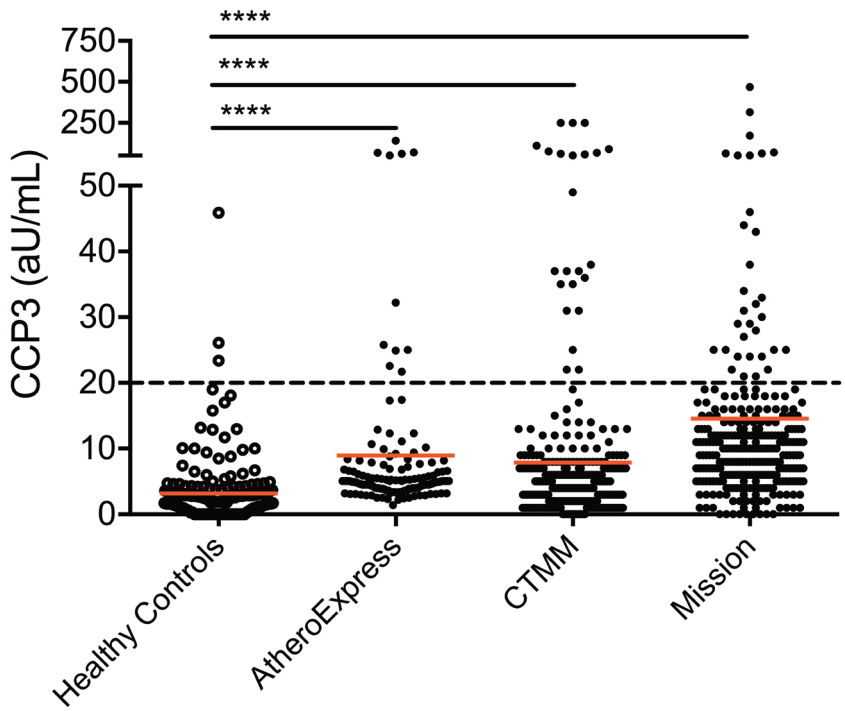

Figure 1. Presence of ACPA by determination of CCP3 reactive antibodies in the sera of age-matched healthy controls and three cohorts of cardiovascular patients. Dashed line indicates the cut-off value, which was set on 20 aU/mL. **** P=0.0001

Circulating Cells

Clinical analysis of ACPA positivity in the circulating cells cohort showed no significant differences in clinical characteristics between CCP3 negative and positive patients as shown in table 3. However, the majority of the CCP3 positive patients were diagnosed with stable CAD (90%).

Table 4: Characteristics of CCP3 negative and positive CV patients in the Circulating Cells cohort

Characteristic	CCP3 Negative	CCP3 Positive	P value
Age, mean years (SD)	62 (10)	67 (10)	0,174
BMI, mean kg/m2 (SD)	26,9 (4,4)	26 (3)	0,453
Men	301/421 (71%)	13/22 (59%)	0,212
SBP (mm Hg) (IQR)	134 (121-145)	143 (128-158)	0,174
DBP (mm Hg) (IQR)	80 (70-85)	77 (73-82)	0,944
Glucose (mmol/L) (IQR)	6,0 (5,4-6,9)	6,0 (5,6-6,7)	0,687
HB (mmol/L) (IQR)	887 (8,3-9,2)	8,6 (8,4-9,1)	0,742
LDL-C (mmol/L) (IQR)	2,39 (1,88-3,02)	2,20 (1,81-2,63)	0,350
HDL-C (mmol/L) (IQR)	1,04 (0,89-,1,23)	1,03 (0,94-1,37)	0,730
Triglycerides (mmol/L) (IQR)	1,32 (1,00-1,91)	1,05 (0,84-1,62)	0,080
hsCRP (ng/mL) (IQR)	2954 (1672-7281)	3997 (2033-7134)	0,381
WBC (x1000 cells/µL) (IQR)	7,1 (5,9-8,5)	7,3 (6,2-8,9)	0,549
Current smoker	92/421 (22%)	5/22 (23%)	0,928
Diabetes Mellitus	86/421 (21%)	4/22 (18%)	0,790
Hypertension	265/421 (65%)	16/22 (73%)	0,360
Beta-Blocker	317/421 (75%)	19/22 (86%)	0,237
Ca-antagonist	123/421 (29%)	7/22 (32%)	0,794
Aspirin	353/421 (84%)	16/22 (73%)	0,173
Vitamin K antagonist	44/421 (10%)	3/22 (14%)	0,636
Statin	334/421 (79%)	17/22 (77%)	0,816

MISSION!

CCP3 positivity did not associate with clinical parameters within the MISSION! as shown in table 2.

Table 5: Characteristics of CCP3 negative and positive CV patients in the MISSION! cohort

Characteristic		CCP3 Negative	CCP3 Positive	P value
		n= 246	N = 29	
Patient characteristics				
Age, mean (SD), y		58,4 (11,6)	62,7 (10,4)	0,056
Woman		55 (22)	10 (34)	0,146

Characteristic		CCP3 Negative	CCP3 Positive	P value
Body mass index, mean (SD), kg/m2		26,5 (4,1)	27,0 (5,5)	0,586
Risk factors				
Treated hypertension		70 (28)	6 (21)	0,376
Diabetes		23 (9)	2 (7)	0,664
Treated hyperlipidemia		44 (18)	4 (14)	0,583
Current smoker		136 (55)	16 (55)	0,991
Family history of CVD		108 (44)	14 (48)	0,667
Clinical characteristics				
Out of hospital cardiac arrest		7 (3)	0 (0)	0,355
Cardiogenic shock		5 (2)	0 (0)	0,445
Anterior infarction		128 (55)	17 (63)	0,413
Infarct size, median (IQR), g/m2		8,6 (4,4-15,4)	13,1 (4,7-18,7)	0,250
Troponine max, median (IQR)		5,5 (2,4-9,5)	8,6 (2,4-14,1)	0,126
CK max, median (IQR)		1948 (957-3512)	2297 (1045-4657)	0,258
Troponine max (log)		0,60 (0,55)	0,78 (0,52)	0,129
CK max (log)		3,2 (0,5)	3,3 (0,5)	0,264
Number of vessels diseased (>50%)	1	141 (58)	13 (45)	0,183
	2	92 (38)	15 (52)	0,144
	3	11 (5)	1 (3)	0,792
Complete revascularization		244 (100)	29 (100)	~
Culprit vessel	RCA	74 (30)	8 (28)	0,781
	LAD	133 (54)	17 (59)	0,641
	LCX	39 (16)	4 (14)	0,773
Proximal lesion		126 (51)	12 (41)	0,316
TIMI flow before intervention 0		169 (69)	20 (71)	0,814
Drug-eluting stent		124 (50)	11 (38)	0,204
Irreversible ischemia myoview		178 (78)	20 (80)	0,824
Irreversible ischemia myoview		96 (43)	11 (46)	0,780
Syntax score before pPCI (IQR)		14,5 (9,0-20,5)	14,3 (7,3-19,9)	0,418
Syntax score after pPCI (IQR)		9,0 (7,0-13,0)	8,0 (4,3-14,5)	0,389
CRP, median (IQR)		0,0 (0,0-6,0)	5,0 (0,0-9,0)	0,122
CRP (log)		(-)4,8 (5,5)	(-)3,1 (5,4)	0,124
CRP >3mg/L		92 (43)	15 (60)	0,110
Clinical endpoints				
Re-infarct		32 (13)	4 (14)	0,906
Cardiac death		9 (4)	4 (14)	0,031
Death		27 (11)	8 (28)	0,011
Re-infarct or death		54 (22)	11 (38)	0,055

Using the MISSION! cohort we were able to investigate whether CCP3 positivity correlated with long term mortality, which is of interest, as it is known from RA patient studies that ACPA positivity increases the overall mortality rate. As shown in figure 2 there was an association between ACPA positivity and mortality rate after 10 years of follow-up. One possibility could be that the atherosclerotic lesion complexity, location and number of affected coronary arteries is dependent on ACPA levels. These parameters are used to calculate the SYNTAX score. We determined the SYNTAX score of all included patients in the MISSION! cohort. We did not observe a correlation between ACPA levels and SYNTAX score (data not shown).

Discussion

The pathogenesis of RA is characterized by the development of different autoreactive antibodies such as rheumatoid factor (RF), anti-carbamylated proteins antibodies (a-CarP) and anti-citrullinated protein antibodies (ACPA) [2, 19, 20]. Especially ACPA is an important clinical biomarker, which is strongly associated with a more progressive disease course, response to treatment and degree of joint destruction [2]. In addition, the presence of ACPA in RA patients is associated with increased incidence of cardiovascular disease and mortality [21]. Products of persistent inflammation in RA, e.g. cytokines and

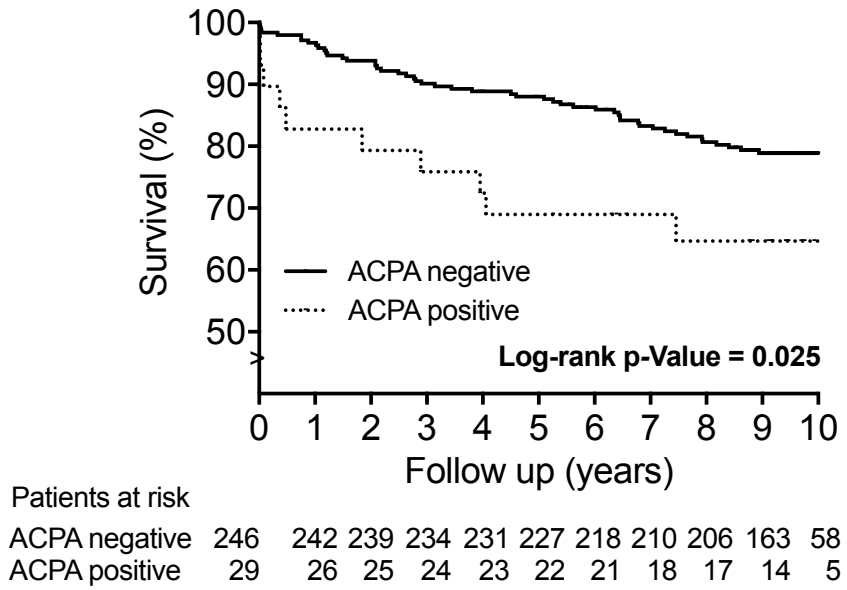

Figure 2. Increased cumulative cardiac mortality was observed in ACPA positive patients compared to ACPA negative patients. Corrected for age, ACPA positivity was independently associated with long-term mortality [HR 2.4 (CI 1.1-5.4) p-Value= 0.025].

antibodies, are thought to directly influence the progression of atherosclerosis [22]. Both RA and atherosclerosis share similar immunological pathways and therefore, presence of ACPA may also affect the vascular inflammation. For example, innate immune cells (macrophages, neutrophils and mast cells) with ACPA bound to their Fcγ receptors can get further activated upon interaction of cell-surface bound ACPA with citrullinated antigens [23–25]. To date, several studies have shown the presence of citrullinated proteins inside atherosclerotic lesions and within the myocardial tissue of RA as well as non-RA patients [7, 26]. In addition, enzymes that drive the citrullination process, PAD enzymes, are also known to be present inside the plaque [8]. Taken together, ACPA mediated activation of local intraplaque immune cells could lead to the release of pro-inflammatory mediators such as cytokines, chemokines and proteases. In turn, this will further enhance the lipid accumulation and destabilization of the atherosclerotic lesion.

In this study, we showed that ACPA can be detected in the serum of a subpopulation of CV patients, who had no history of rheumatic diseases. We screened three cohorts; AtheroExpress, Circulating Cells and MISSION! for the presence of ACPA. In all three tested cohorts we found that a significant proportion (5-10%) of the CV patients showed reactivity towards citrullinated peptides (CCP3) compared to 1% positivity in healthy age-matched controls. This observation is in line with a previous study, which reported presence of ACPA in 10,4% of the cases in a cohort of coronary heart disease patients compared to 3,8% in controls [9]. These data indicate that an humoral immune response towards citrullinated proteins can not only develop in RA, but also in a chronic systemic inflammatory diseases such as atherosclerosis.

In contrast to RA, the pathogenic contribution of autoreactive antibodies in cardiovascular diseases is still a subject of debate. On one hand, IgM producing B1 B cells have shown to be atheroprotective, while on the other hand IgG producing B2 B cells are reported to aggravate atherosclerosis [27]. Next to antibodies, B cells produce a variety of mediators and are able to present antigens to T cells. This underscores that B cells and their products can significantly contribute to the pathogenesis of atherosclerosis and RA. Indeed, B cell depleting therapies, such as the use of rituximab, in RA have been shown to be effective and these studies have also shown beneficial effects on the progression of atherosclerosis. Treatment of RA patients with rituximab for a period of 4 months showed an improvement in lipid profiles and a reduction in carotid intima- media thickness [28]. In addition, improvements in endothelial function as determined by flow- mediated vasodilation and in systemic inflammation as indicated by serum levels of hsCRP were reported in another clinical study, in which RA patients were treated with rituximab [29]. These studies underline a significant role for B cells in vascular inflammation in active RA patients, which is mediated by the secretion of cytokines and antigen presentation and/or the production of auto- reactive antibodies.

Association analyses of ACPA positivity in cardiovascular patients with parameters such as lipid profile, inflammatory markers and medication use did not reveal any associations in

all three analyzed cohorts. We compared plaque phenotype of ACPA positive CV patients of the AtheroExpress cohort, which did not reveal any differences in vulnerable plaque characteristics between ACPA positive and negative patients. In RA, the presence of ACPA is associated with increased mortality [30, 31]. Also in non-rheumatic cardiovascular patients of the MISSION! cohort we observed a significant association between ACPA positivity and long term mortality. We did not investigate the causal mechanism in this current study but one possibility could be the accelerated atherosclerosis in ACPA positive patients. We further analyzed this by characterizing the complexity of the coronary arteries disease by SYNTAX scoring the patients in the MISSION! cohort. However, this analysis did not reveal an association between SYNTAX score and ACPA positivity. Further research could focus on investigating the systemic inflammatory status of these patients by determining circulating proatherogenic mediators. In addition, the intraplaque citrullination could be different between APCA negative and positive patients, which could also influence the plaque stability.

There are several limitations to our study. The reported APCA titers in the presented cohorts are relatively low compared to the levels in established RA patients. This could indicate that there is a difference in the development of ACPA producing B cells and/or the inflammatory milieu in cardiovascular versus RA patients. Further research should focus on the comparison of cardiovascular ACPA with RA ACPA in terms of glycosylation, fine specificity and avidity as well as isotype class. In addition, we used a commercially available kit to determine ACPA levels. Although this assay is a highly sensitive and reproducible method, we were unable to control for citrulline specificity by including an arginine control. Finally, although we excluded all patients with known rheumatic diseases, we cannot exclude that these ACPA positive CV patients will develop symptoms of RA in the future.

In conclusion, here we confirm the presence of ACPA in serum of a subpopulation of CV patients. Analysis showed an association between ACPA positivity and long-term mortality. Further research should focus on characterizing the phenotype of cardiovascular ACPA compared to rheumatic ACPA in terms of isotype, glycosylation and affinity towards citrullinated proteins.

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Ann Rheum Dis. doi: 10.1136/annrheumdis-2015-208579