Rheumatol Int (2015) 35:1541–1547
DOI 10.1007/s00296-015-3244-y

Rheumatology INTERNATIONAL

ORIGINAL ARTICLE - OBSERVATIONAL RESEARCH

Serum levels of C-peptide are associated with coronary artery calcification in patients with rheumatoid arthritis

Mathias Burgmaier[1,7] · Sophia Hoppe[2] · Thilo Krüger[3,7] · Andreas H. Mahnken[4] · Markus Ketteler[5] · Sebastian Reith[1] · Georg Mühlenbruch[6] · Nikolaus Marx[1] · Vincent Brandenburg[1]

Received: 20 October 2014 / Accepted: 3 March 2015 / Published online: 18 March 2015

Abstract C-peptide has pro-atherogenic effects in animal models, and elevated C-peptide levels are associated with cardiovascular and all-cause mortality in patients undergoing coronary angiography. This cross-sectional study investigated the association between C-peptide serum levels and coronary artery calcification (CAC) in patients with rheumatoid arthritis (RA), a high-risk group for cardiovascular events. Fifty-four patients with RA were recruited from an arthritis outpatient department at the University Hospital in Aachen, Germany. CAC was measured by multi-slice CT scan, and blood samples were drawn from all patients for the analysis of C-peptide and other cardiovascular biomarkers. Mean serum levels of C-peptide (1.187 ± 0.771 vs 0.745 ± 0.481 nmol/L, $p = 0.02$), YKL-40, LDL cholesterol, and triglycerides were significantly higher in patients with CAC ($n = 32$, 59 %) compared to those without CAC ($n = 22$, 41 %). Univariate analysis revealed a significant association of C-peptide [OR 4.7, 95 % CI (1.1, 20.2)], YKL-40, triglycerides, hypertension, smoking, age, and male sex with the presence of CAC. After adjustment for body mass index, cholesterol, diabetes, adiponectin, calcium, and phosphate, C-peptide was still significantly associated with CAC in a multivariate logistic regression model. In conclusion, C-peptide serum levels are independently associated with the presence of CAC in patients with RA. These data suggest a potential role of C-peptide in cardiovascular disease in patients with RA.

Keywords Coronary artery calcification · Atherosclerosis · C-peptide · Rheumatoid arthritis

✉ Nikolaus Marx
nmarx@ukaachen.de

1 Department of Internal Medicine I – Cardiology, University Hospital of the RWTH Aachen, Pauwelstr 30, 52074 Aachen, Germany

2 Department of Neurology, University Hospital of the RWTH Aachen, Aachen, Germany

3 Department of Internal Medicine II, University Hospital of the RWTH Aachen, Aachen, Germany

4 Department of Diagnostic and Interventional Radiology, University Hospital, Philipps-University Marburg, Marburg, Germany

5 Department of Nephrology, Klinikum Coburg, Coburg, Germany

6 Department of Neuroradiology, University Hospital of the RWTH Aachen, Aachen, Germany

7 IZKF, University Hospital of the RWTH Aachen, Aachen, Germany

Introduction

C-peptide—initially thought to be biologically inert—is produced by cleavage from proinsulin [1]. Work from our laboratory and others could demonstrate that C-peptide may influence several critical steps in atherogenesis. Specifically, C-peptide co-localizes with macrophages in early atherosclerotic lesions and increases migration of human monocytes [2] as wells as human CD4-positive lymphocytes [3, 4]. Furthermore, C-peptide enhances the proliferation of vascular smooth muscle cells [5]. In ApoE$^{-/-}$ mice, subcutaneous application of C-peptide promotes atherosclerosis development as well as plaque inflammation [6]. Recently, C-peptide has been shown to be independently associated with cardiovascular mortality in patients undergoing coronary angiography [7]. Taken together, these studies suggest that C-peptide may play a role in human atherosclerosis.

Current data suggest that atherosclerosis is an inflammatory process in the vessel wall [8]. Therefore, pro-inflammatory mediators and biomarkers, such as high-sensitive C-reactive protein, fibrinogen, and YKL-40, have been shown to be associated with progressive cardiovascular disease and an increased risk of cardiovascular morbidity and mortality [9–11]. However, being part of the acute phase reaction, these biomarkers lack specificity especially in patients with a systemic inflammatory response. There is an ongoing debate whether systemic inflammation and corresponding inflammatory markers trigger inflammatory processes in the vessel wall or vice versa. Therefore, there is a need for novel, specific cardiovascular biomarkers which are independent of the current systemic inflammatory response of patients. C-peptide may be a potential candidate since its serum levels are mainly regulated by insulin secretion and kidney function [12].

Disease conditions with chronic inflammation such as rheumatoid arthritis (RA) are characterized by accelerated and excessive cardiovascular disease [13–15]. Given the high prevalence of coronary artery disease in patients with RA even in the absence of classical cardiovascular risk factors [16] and since C-peptide has previously been shown to be associated with cardiovascular mortality [7], this study aimed to investigate the association of C-peptide serum levels with CAC in the high-risk group of patients with RA.

Methods

For this cross-sectional study, 54 patients with RA were recruited from a RA outpatient department associated with the University Hospital in Aachen, Germany. Patients underwent both multi-slice computed tomography scanning (MSCT) for CAC as well as laboratory testing. In order to prevent selection bias, patients presenting themselves to the outpatient department were recruited consecutively in case they met the following inclusion criteria: a minimum of 5 years RA duration, age >30 years, and informed consent to the study protocol. All patients were eligible for the study. Exclusion criteria were pregnancy, immobilization, severe comorbidities requiring medical assistance for transport and performance of multi-slice CT scanning, as well as cancer, acute viral or bacterial infections, atrial fibrillation, a history of coronary artery bypass grafting, aortic/mitral valve operation or percutaneous transluminal coronary angioplasty as well as claustrophobia. Three patients had to be excluded from the study due to atrial fibrillation ($n = 1$) and aortic/mitral valve operation ($n = 2$) after preliminary consent.

Patient-reported outcomes for RA regarding functional capacity were documented by the Hannover Functional Questionnaire [Funktionsfragebogen Hannover (FFbH)]—a questionnaire resembling the Health Assessment Questionnaire [17]. The FFbH describes the functional capacity to perform daily-life activities in percentage, with 100 % indicating no and 0 % indicating maximal limitations.

Clinical cardiovascular risk factors were assessed by chart review as well as patient history taken at the time of MSCT scanning. Arterial hypertension was defined as the usage of antihypertensive drugs or an arterial blood pressure exceeding 135/85 mmHg. Renal function was assessed via the estimated glomerular filtration rate (GFR) calculation based on the MDRD formula [18].

The presence of diabetes was assessed via chart review and patient interview: Diabetes was diagnosed if the patient reported the diagnosis, if diabetes was listed in the medical history, and if antidiabetic drugs were prescribed.

Imaging procedure

All patients underwent cardiac multi-slice CT scanning by a 16-slice multi-slice CT scanner (SOMATOM, Sensation 16, Siemens, Forchheim, Germany). Scan parameters included a collimation of 12 × 0.75 mm, a rotation time of 420 ms, a table feed of 3.4 mm per rotation, a tube voltage of 120 kV, and an effective tube current time product of 150 mAseff. For ECG synchronization, retrospective ECG gating was applied. Axial images were reconstructed in mid-diastole at 60 % of the RR interval with an effective slice thickness of 3 mm and a reconstruction increment of 2 mm. A dedicated convolution kernel (B35f), a field of view of 180 × 180 mm^2, and a matrix of 512 × 512 were applied. All multi-slice CT images were assessed by an experienced radiologist with 5 years of training in cardiac imaging. Image analysis was performed on a separate computer workstation (Leonardo, Siemens, Forchheim, Germany) equipped with a dedicated software tool for calcium scoring (Calcium Scoring CT, Siemens, Forchheim, Germany). CAC scores were calculated according to the method originally described by Agatston et al. [19].

Biochemical analysis

Serum creatinine, C-reactive protein (CRP), total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides were all measured via standard laboratory methods in laboratories associated with the clinic from which patients were included.

For centralized laboratory measurements, serum was harvested after fasting overnight on the day of the multi-slice CT scanning and immediately frozen (−20 °C). Afterward, samples were transferred to the University Hospital Aachen for long-term storage at −80 °C. The following parameters were all measured centrally after study end using commercially available kits according to the manufacturer's protocol:

Adiponectin, YKL-40, and C-peptide. All measurements were taken by a person blinded to the study protocol, and each analysis was performed with an independent aliquot in order to avoid any freeze–thaw cycle prior to the analysis.

For YKL-40 measurement, we used the MicroVue™ YKL-40 enzyme immunoassay by Quidel, San Diego, USA, and for the determination of adiponectin levels, the TECO Adiponectin enzyme immunoassay, TECOmedical, Sissach, Switzerland. C-peptide serum levels were determined by the Mercodia C-peptide ELISA, Mercodia AB, Uppsala, Sweden, according to the manufacturer's protocol. This C-peptide assay has been previously used to analyze C-peptide levels in clinical studies [20]. Briefly, C-peptide in the sample reacts with anti-C-peptide antibodies bound to the microtitration well. After washing, peroxidase-conjugated anti-C-peptide antibodies are added, and after the second incubation and washing step, the bound conjugate is detected by reaction with 3,3′,5,5′-tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at 450 nm.

Statistical analysis

To investigate the association between biomarkers and coronary artery disease, patients were divided into two groups according to the absence (Agatston score = 0, $n = 22$) or the presence (Agatston score > 0, $n = 32$) of coronary artery disease [19]. Continuous factors are expressed as mean values and standard deviation (SD). Categorical factors are presented as frequencies and percentage. Differences between groups were calculated with *t* test (continuous variables) or Pearson's Chi-squared test (nominal parameters).

To investigate the prognostic value of biomarkers and potential associated factors for CAC, univariate logistic regression analysis was performed. Using multivariate logistic regression analysis, adjustments for multiple cardiovascular associated factors and biomarker were performed in order to elucidate confounding variables and to investigate independent associations of C-peptide with the presence of CAC. A p value <0.05 was regarded as statistical significance.

Test results are reported as p values (p), odds ratio, and corresponding 95 % confidence interval (CI). The above analyses were performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Besides the presence of RA (100 %) and arterial hypertension (61 %), the study population was characterized by a low prevalence of classical cardiovascular risk factors. Only $n = 4$ patients (7 %) had type 2 diabetes mellitus and 15 % were active smokers. None of the patients was diagnosed with type 1 diabetes mellitus.

Whereas chronic kidney disease (CKD) stage II was common (48 %), only 6 % had CKD stage III and none (0 %) exhibited CKD stage IV or end-stage renal failure. Patients had a mean body mass index (BMI) of 26.9 ± 5.7 kg/m^2, with 39 (72 %) being normal weight or overweight (BMI > 25) and 14 (26 %) being obese (BMI > 30.0, Table 1). The majority of patients was female (72 %), and mean age was 58 ± 10 years (31–72 years). Further patient characteristics as well as immunosuppressive/antirheumatic therapy at the time of blood drawing are shown in Table 1.

Intergroup comparison in patients with and without CAC: anthropomorphic data

To investigate the association between coronary artery disease and C-peptide as well as other cardiovascular biomarkers, patients were divided into two groups according to the absence (Agatston score = 0, $n = 22$) or the presence (Agatston score > 0, $n = 32$) of CAC [19]. The mean Agatston score of patients with coronary artery disease was 219 ± 365 (Table 2). The majority of patients with CAC had mild CAC [Agatston score < 100, $n = 20$ (62.5 %)], whereas $n = 12$ (37.5 %) had moderate and severe calcification (Agatston score > 100, Table 1).

As shown in Table 2, patients with CAC were significantly older ($p < 0.0001$) compared to those without CAC (62 ± 6 vs 52 ± 11 years, respectively). Among patients with CAC, $n = 14$ (44 %) were male, and only one man (5 %) exhibited no CAC.

Intergroup comparison in patients with and without CAC: classical cardiovascular risk factors

The extent of smoking (15.4 ± 19.6 vs 3.0 ± 5.6 pack-years) was significantly higher in patients with CAC compared to those without CAC ($p < 0.01$, Table 2). Among patients with CAC, $n = 24$ (75 %) had arterial hypertension compared to $n = 9$ (41 %) subjects without CAC. The overall prevalence of obesity, diabetes mellitus, and CKD was low (Table 1), and there was no significant difference with respect to BMI and GFR in patients with compared to those without CAC (p = ns, Table 2).

Moreover, there was no significant difference between patients without and with CAC regarding rheumatoid disease duration as well as clinical severity of RA as determined by the Hannover Functional Questionnaire (FFbH, Table 2).

Table 1 Patient characteristics of 54 patients with rheumatoid arthritis

	$N = 54$
Anthropomorphic data	
Male gender (*n*, %)	15 (27.8 %)
Age (years)	57.91 ± 9.73
Classical cardiovascular risk factors	
Smoking	
Active (*n*, %)	8 (14.8 %)
Non-smoking (*n*, %)	32 (59.3 %)
Former smoking (*n*, %)	14 (25.9 %)
Pack-years (years)	10.37 ± 16.61
Hypertension (*n*, %)	33 (61.1 %)
Systolic blood pressure (mmHg)	134 ± 18
Diastolic blood pressure (mmHg)	84 ± 10
Diabetes mellitus (*n*, %)	4 (7.4 %)
Previous cardiovascular disease (*n*, %)	18 (33.3 %)
BMI (kg/m^2)	26.91 ± 5.66
<18.5 kg/m^2 (*n*, %)	1 (1.9 %)
18.5–24.9 kg/m^2 (*n*, %)	19 (35.2 %)
25.0–29.9 kg/m^2 (*n*, %)	20 (37.0 %)
>30.0 kg/m^2 (*n*, %)	14 (26.0 %)
GFR (ml/min/1.73 m^2)	89.60 ± 20.52
CKD II (*n*, %)	26 (48.1 %)
CKD III (*n*, %)	3 (5.6 %)
CKD IV or V (*n*, %)	0 (0 %)
FFbH (%)	71.14 ± 25.31
Disease duration (years)	16.83 ± 7.81
RF positivity (*n*, %)	41 (75.9 %)
ANA positivity (*n*, %)	12 (22.2 %)
Cardiovascular biomarker	
Total cholesterol (mg/dL)	213 ± 42
LDL cholesterol (mg/dL)	115 ± 34
HDL cholesterol (mg/dL)	74 ± 24
Triglyceride (mg/dL)	143 ± 104
Adiponectin (mg/dL)	12 ± 7
Calcium (mmol/L)	2.36 ± 0.16
Phosphate (mmol/L)	1.01 ± 0.21
CRP (mg/dL)	5.8 ± 7
YKL-40 (mg/dL)	125 ± 83
C-Peptide (nmol/L)	1.005 ± 0.697
Major drug classes	
Statins (*n*, %)	9 (16.7 %)
Antihypertensive (*n*, %)	18 (33.3 %)
Steroid (*n*, %)	45 (83.3 %)
MTX (*n*, %)	31 (57.4 %)
Chloroquine (*n*, %)	17 (31.5 %)
Hydroxychloroquine (*n*, %)	19 (35.2 %)
Leflunomide (*n*, %)	29 (53.7 %)
Gold (*n*, %)	25 (46.3 %)
Sulfasalazine (*n*, %)	37 (68.9 %)
Azathioprine (*n*, %)	15 (27.8 %)
Calcification	
Agatston score = 0 (*n*, %)	22 (40.7 %)
Agatston score = 0–10 (*n*, %)	8 (14.8 %)
Agatston score = 10–100 (*n*, %)	12 (22.2 %)
Agatston score = 100–400 (*n*, %)	7 (13.0 %)
Agatston score > 400 (*n*, %)	5 (9.3 %)

BMI body mass index, *GFR* glomerular filtration rate, *RF* rheumatoid factor, *ANA* antinuclear antibodies, *CKD* chronic kidney disease, *FFbH* Functional Questionnaire Hannover for rheumatoid arthritis, *MTX* methotrexate

Intergroup comparison in patients with and without CAC: lipid profile and adiponectin levels

LDL cholesterol (125 ± 26 vs 101 ± 40 mg/dL, $p = 0.01$) as well as triglyceride levels (174 ± 122 vs 97 ± 36 mg/dL, $p < 0.01$) were significantly increased in patients with CAC compared to those without CAC, while there was no difference in HDL or total cholesterol (p = ns). Furthermore, there was no difference in adiponectin levels in patients without CAC compared to those with CAC (p = ns).

Intergroup comparison in patients with and without CAC: cardiovascular biomarkers

C-peptide serum levels were significantly higher in patients with CAC compared to those without CAC (1.187 ± 0.771 vs 0.745 ± 0.481 nmol/L, $p = 0.02$). There was no difference in CRP levels in patients without CAC compared to those with CAC (5.70 ± 5.62 vs 5.90 ± 8.52 mg/L, p = ns). However, the cardiovascular and inflammatory biomarker YKL-40 was significantly increased in patients with CAC compared to those without CAC (150 ± 85 vs 89 ± 67 ng/mL, $p < 0.01$).

Uni- and multivariate associated factors analysis for CAC in patients with rheumatoid arthritis

Uni- and multivariate regression analysis was performed to identify associated factors for the presence of CAC. Univariate analysis revealed significant contributions of C-peptide [OR 4.689, 95 % CI (1.084, 20.227)], YKL-40, triglycerides, hypertension, smoking, age, and male sex (all $p < 0.05$, Table 3) with the presence of CAC.

Since C-peptide was associated with the presence of CAC, levels of C-peptide were adjusted for multiple cardiovascular risk factors and biomarkers such as epidemiological parameters (age, gender; Table 4, model 1), kidney function (GFR; Table 4, model 2), inflammatory and

Table 2 Intergroup comparison according to the absence or the presence of coronary artery calcifications (CAC)

	CAC = 0 $N = 22$	CAC > 0 $N = 32$	*p* value
Anthropomorphic data			
Male gender (*n*, %)	1 (5 %)	14 (44 %)	<0.01
Age (years)	52 ± 11	62 ± 6	<0.0001
Cardiovascular risk factors			
Smoking (pack-years)	3.0 ± 5.6	15.4 ± 19.6	<0.01
Hypertension (*n*, %)	9 (41 %)	24 (75 %)	0.01
Diabetes mellitus (*n*, %)	1 (5 %)	3 (9 %)	ns
BMI (kg/m^2)	26.02 ± 6.84	27.51 ± 4.7	ns
GFR (ml/min/1.73 m^2)	91.43 ± 24.12	88.36 ± 18.00	ns
Time of RA (years)	16.32 ± 8.58	17.17 ± 7.37	ns
FFbH (%)	75.76 ± 24.73	67.97 ± 25.01	ns
Cardiovascular biomarker			
Total cholesterol (mg/dL)	200.18 ± 40.42	222.31 ± 40.53	ns
LDL cholesterol (mg/dL)	101.25 ± 39.84	125.14 ± 26.04	0.01
HDL cholesterol (mg/dL)	80.01 ± 26.54	70.46 ± 22.00	ns
Triglyceride (mg/dL)	97.34 ± 35.86	174.18 ± 122.48	<0.01
Adiponectin (µg/mL)	12.44 ± 6.50	11.58 ± 7.72	ns
CRP (mg/L)	5.70 ± 5.62	5.90 ± 8.52	ns
YKL-40 (ng/mL)	88.54 ± 66.83	149.76 ± 84.99	<0.01
C-Peptide (nmol/L)	0.745 ± 0.481	1.187 ± 0.771	0.02
Agatston score (score points)	0 ± 0	219 ± 365	

BMI body mass index, *GFR* glomerular filtration rate, *FFbH* Functional Questionnaire Hannover for rheumatoid arthritis

cardiovascular biomarkers (CRP, YKL-40; Table 4, model 2), parameters of lipid metabolism (cholesterol, adiponectin, BMI; Table 4, model 3), parameters of calcification (calcium and phosphate; Table 4, model 4), and the presence of diabetes (Table 4, model 3 and 4). This was calculated in several models in order to avoid overadjustment and to investigate whether C-peptide was independently associated with CAC (Table 4, models 1–4). These multivariate logistic regression models revealed that C-peptide was a significant predictor for the presence of CAC (all $p < 0.05$).

Discussion

In this study, the present data demonstrate an association between circulating C-peptide levels and the presence of coronary calcification in patients with RA.

The proinsulin cleavage product C-peptide is secreted into the blood stream in amounts equimolar to insulin [21]. Experimental data suggest that C-peptide may exhibit proatherogenic effects, and recent clinical data could show that C-peptide is independently associated with cardiovascular mortality in patients undergoing coronary angiography [7], suggesting a direct role of C-peptide in human atherosclerosis. The present study extends the current knowledge by demonstrating that C-peptide levels are associated with the presence of CAC as determined by CT scan in high-risk patients with RA.

Atherosclerotic plaque burden and plaque properties cannot be analyzed noninvasively. Since the extent of CAC quantification has been demonstrated to correlate with the overall atherosclerotic burden, we assessed CAC via MSCT, the most common imaging tool for arterial wall examination [22].

Atherosclerosis is thought to be an inflammatory process within the vessel wall [8]. As such, a large quantity of proinflammatory parameters have been suggested as cardiovascular biomarkers [23]. However, being part of the acute phase reaction, many of these biomarkers lack specificity especially in patients with a systemic inflammatory disease such as RA.

Therefore, we investigated the role of various cardiovascular biomarkers in patients with RA, a high-risk group for coronary artery disease with a chronic high state of inflammation. In the present study, we observed no difference in CRP levels between patients with and without CAC. As expected in RA patients, CRP levels were slightly to moderately elevated in this study population. However, YKL-40, thought to play a role in both atherogenesis and RA [10, 24, 25], was elevated in patients with CAC and was a predictor for the presence of CAC in our univariate logistic regression analysis. Future studies are needed to assess the association between increased C-peptide levels, metabolic syndrome, disturbed insulin sensitivity or subclinical diabetes, and coronary atherosclerosis.

The present study has several limitations. Although our data were adjusted for diabetes mellitus, we cannot exclude that some patients were insulin-resistant since, e.g., oral glucose tolerance testing was not performed and insulin levels were not determined due to shortage of specimen. Thus, we cannot comment on the contribution of subclinical diabetes to the present association between C-peptide and CAC. This may particularly be of importance since recent data suggest that non-diabetic patients with RA exhibit impaired beta cell function compared to controls [26]. Furthermore, the sample size of this study is small and the findings need to be confirmed in larger studies.

In summary, this study shows an association between C-peptide and CAC in patients with RA. Future prospective

Table 3 Univariate analysis for the presence of CAC

Parameter	Odds ratio	95 % confidence interval		*p* value
		Lower	Upper	
Anthropomorphic data				
Female gender (present)	0.061	0.007	0.512	0.01
Age (years)	1.148	1.058	1.245	<0.001
Cardiovascular risk factors				
Smoking (pack-years)	1.075	1.011	1.143	0.02
Hypertension (present)	4.333	1.349	13.924	0.01
Diabetes mellitus (present)	0.460	0.045	4.740	ns
BMI (kg/m^2)	1.054	0.945	1.175	ns
GFR (ml/min/1.73 m^2)	0.993	0.966	1.020	ns
Time of RA (years)	1.014	0.945	1.089	ns
FFbH (%)	0.987	0.964	1.010	ns
Cardiovascular biomarker				
Total cholesterol (mg/dL)	1.014	0.999	1.030	ns
LDL cholesterol (mg/dL)	1.024	1.004	1.045	0.02
HDL cholesterol (mg/dL)	0.983	0.960	1.007	ns
Triglyceride (mg/dL)	1.014	1.002	1.027	0.03
Adiponectin (μg/mL)	0.983	0.909	1.063	ns
CRP (mg/L)	1.004	0.930	1.083	ns
YKL-40 (ng/mL)	1.012	1.002	1.021	0.02
C-Peptide (nmol/L)	4.689	1.084	20.227	0.04

BMI body mass index, *GFR* glomerular filtration rate, *FFbH* Functional Questionnaire Hannover for rheumatoid arthritis

Table 4 Multivariate analysis for the presence of CAC

Parameter	Odds ratio	95 % confidence interval		*p* value
		Lower	Upper	
Model 1: adjusted for sex, age				
C-Peptide (nmol/L)	6.460	1.002	41.646	0.0498
Model 2: adjusted for sex, age, YKL-40, CRP, GFR				
C-Peptide (nmol/L)	8.581	1.135	64.870	0.0373
Model 3: adjusted for BMI, cholesterol, diabetes, adiponectin				
C-Peptide (nmol/L)	4.725	1.169	19.103	0.0293
Model 4: adjusted for BMI, cholesterol, diabetes, adiponectin, calcium, phosphate				
C-Peptide (nmol/L)	6.297	1.192	33.254	0.0302

BMI body mass index, *GFR* glomerular filtration rate

studies are needed to determine whether the association between C-peptide levels and the presence of CAC observed in this study translates into increased cardiovascular events in patients with RA and elevated C-peptide levels.

Acknowledgments This work was supported by a Grant from the Interdisciplinary Centre for Clinical Research (IZKF) within the faculty of medicine at the RWTH Aachen University to PD Dr. Mathias Burgmaier and Dr. Thilo Krueger.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Marx N, Walcher D (2008) C-Peptide and atherogenesis: C-Peptide as a mediator of lesion development in patients with type 2 diabetes mellitus? Exp Diabetes Res 2008:385108
2. Marx N, Walcher D, Raichle C, Aleksic M, Bach H, Grub M, Hombach V, Libby P, Zieske A, Homma S, Strong J (2004) C-peptide colocalizes with macrophages in early arteriosclerotic lesions of diabetic subjects and induces monocyte chemotaxis in vitro. Arterioscler Thromb Vasc Biol 24(3):540–545
3. Aleksic M, Walcher D, Giehl K, Bach H, Grub M, Durst R, Hombach V, Marx N (2009) Signalling processes involved in C-peptide-induced chemotaxis of CD4-positive lymphocytes. Cell Mol Life Sci 66(11–12):1974–1984
4. Walcher D, Aleksic M, Jerg V, Hombach V, Zieske A, Homma S, Strong J, Marx N (2004) C-peptide induces chemotaxis of human CD4-positive cells: involvement of pertussis toxin-sensitive G-proteins and phosphoinositide 3-kinase. Diabetes 53(7):1664–1670
5. Walcher D, Babiak C, Poletek P, Rosenkranz S, Bach H, Betz S, Durst R, Grub M, Hombach V, Strong J, Marx N (2006) C-Peptide induces vascular smooth muscle cell proliferation: involvement of SRC-kinase, phosphatidylinositol 3-kinase, and extracellular signal-regulated kinase 1/2. Circ Res 99(11):1181–1187
6. Vasic D, Marx N, Sukhova G, Bach H, Durst R, Grub M, Hausauer A, Hombach V, Rottbauer W, Walcher D (2012) C-peptide promotes lesion development in a mouse model of arteriosclerosis. J Cell Mol Med 16(4):927–935
7. Marx N, Silbernagel G, Brandenburg V, Burgmaier M, Kleber ME, Grammer TB, Winkelmann BR, Boehm BO, Marz W (2013) C-peptide levels are associated with mortality and cardiovascular mortality in patients undergoing angiography: the LURIC study. Diabetes Care 36(3):708–714. doi:10.2337/dc12-1211
8. Ross R (1999) Atherosclerosis–an inflammatory disease. N Engl J Med 340(2):115–126
9. Corrado E, Rizzo M, Coppola G, Fattouch K, Novo G, Marturana I, Ferrara F, Novo S (2010) An update on the role of markers of inflammation in atherosclerosis. J Atheroscler Thromb 17(1):1–11
10. Kastrup J, Johansen JS, Winkel P, Hansen JF, Hildebrandt P, Jensen GB, Jespersen CM, Kjoller E, Kolmos HJ, Lind I, Nielsen H, Gluud C (2009) High serum YKL-40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease. Eur Heart J 30(9):1066–1072
11. Turkyilmaz AK, Devrimsel G, Kirbas A, Cicek Y, Karkucak M, Capkin E, Gokmen F (2013) Relationship between pulse wave velocity and serum YKL-40 level in patients with early rheumatoid arthritis. Rheumatol Int 33(11):2751–2756. doi:10.1007/s00296-013-2810-4
12. Cahill GF Jr (1973) C-peptide: a new method of assessing pancreatic beta-cell function. N Engl J Med 288(22):1181–1182
13. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, D'Agostino RB, Kuller LH (1997) Age-specific incidence rates of myocardial infarction and angina in

women with systemic lupus erythematosus: comparison with the Framingham Study. Am J Epidemiol 145(5):408–415
14. Jacobsson LT, Knowler WC, Pillemer S, Hanson RL, Pettitt DJ, Nelson RG, del Puente A, McCance DR, Charles PH, Bennett MA (1993) Rheumatoid arthritis and mortality. A longitudinal study in Pima Indians. Arthritis Rheum 36(8):1045–1053
15. Miller AM, McInnes IB (2011) Cytokines as therapeutic targets to reduce cardiovascular risk in chronic inflammation. Curr Pharm Des 17(1):1–8
16. Wang S, Yiu KH, Mok MY, Ooi GC, Khong PL, Mak KF, Lau CP, Lam KF, Lau CS, Tse HF (2009) Prevalence and extent of calcification over aorta, coronary and carotid arteries in patients with rheumatoid arthritis. J Intern Med 266(5):445–452
17. Zochling J, Stucki G, Grill E, Braun J (2007) A comparative study of patient-reported functional outcomes in acute rheumatoid arthritis. J Rheumatol 34(1):64–69
18. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130(6):461–470
19. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R (1990) Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 15(4):827–832
20. Walhin JP, Richardson JD, Betts JA, Thompson D (2013) Exercise counteracts the effects of short-term overfeeding and reduced physical activity independent of energy imbalance in healthy young men. J Physiol 591(Pt 24):6231–6243. doi:10.1113/jphysiol.2013.262709
21. Tager HS, Emdin SO, Clark JL, Steiner DF (1973) Studies on the conversion of proinsulin to insulin. II. Evidence for a chymotrypsin-like cleavage in the connecting peptide region of insulin precursors in the rat. J Biol Chem 248(10):3476–3482
22. Alexopoulos N, Raggi P (2009) Calcification in atherosclerosis. Nat Rev Cardiol 6(11):681–688
23. Hochholzer W, Morrow DA, Giugliano RP (2010) Novel biomarkers in cardiovascular disease: update 2010. Am Heart J 160(4):583–594
24. Peltomaa R, Paimela L, Harvey S, Helve T, Leirisalo-Repo M (2001) Increased level of YKL-40 in sera from patients with early rheumatoid arthritis: a new marker for disease activity. Rheumatol Int 20(5):192–196
25. Zheng JL, Lu L, Hu J, Zhang RY, Zhang Q, Chen QJ, Shen WF (2010) Increased serum YKL-40 and C-reactive protein levels are associated with angiographic lesion progression in patients with coronary artery disease. Atherosclerosis 210(2):590–595
26. Ferraz-Amaro I, Garcia-Dopico JA, Medina-Vega L, Gonzalez-Gay MA, Diaz-Gonzalez F (2013) Impaired beta cell function is present in nondiabetic rheumatoid arthritis patients. Arthritis Res Ther 15(1):R17. doi:10.1186/ar4149