Complement activation products C5a and sC5b-9 are associated with low-grade inflammation and endothelial dysfunction, but not with atherosclerosis in a cross-sectional analysis: The CODAM study

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Low-grade inflammation is involved in endothelial dysfunction, atherosclerosis and cardiovascular disease (CVD) [1]. The complement system is an ancient inducer of inflammation, which generates various soluble and membrane-bound factors upon activation [2]. Complement C5a and soluble C5b-9 (sC5b-9) are products of the final step of the complement activation cascade (= terminal complement activation). The anaphylatoxin C5a is a soluble mediator, which can activate immune and endothelial cells [3]. sC5b-9 is the circulating form of C5b-9, also called membrane-attack complex, which has been shown to affect endothelial cells in vitro [4]. In humans, C5a and sC5b-9 are increased in acute inflammatory situations and in acute cardiovascular events, where they are thought to mediate ischemia–reperfusion injury [5]. Indeed, in patients with acute or advanced CVD, C5a and sC5b-9 have been associated with adverse cardiovascular outcomes [6–9]. However, it is currently unknown whether C5a and sC5b-9 are also increased in earlier, less advanced stages of the cardiovascular disease process. Therefore, we investigated the associations of plasma C5a and sC5b-9 with systemic low-grade inflammation, endothelial dysfunction, markers of atherosclerosis and with prevalent CVD in a cohort of middle-aged individuals with a moderately increased cardiometabolic risk.

The cohort on diabetes and atherosclerosis Maastricht (CODAM) includes Caucasian individuals with one or more cardiometabolic risk factor. Details on the study design and the measurements have been described elsewhere [10]. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre. All participants gave written informed consent. For complement analysis, aliquots were thawed at 37 °C and subsequently transferred to ice. C5a and sC5b-9 were measured with ELISA (Catalogue number A021 and A029, Quidel, San Diego, USA). Inter-assay coefficients of variation were 5.3% and 11.8%, respectively. For low-grade inflammation, a score was generated by averaging the standardised scores [11] of C-reactive protein, interleukin-6, interleukin-8, tumour necrosis factor-alpha, serum amyloid A, soluble intracellular adhesion molecule-1, ceruloplasmin, and haptoglobin. For endothelial dysfunction, a score was generated by averaging standardised scores of soluble E-selectin, soluble vascular adhesion molecule-1 (VCAM-1), and von Willebrand factor (vWF). Markers of atherosclerosis were carotid intima–media thickness (cIMT) and ankle–arm blood pressure index (AAIx). Prevalent CVD was assessed with self-reports, ECG examinations and/or AAIx < 0.9 [12].

We performed cross-sectional analyses among 537 individuals. Skewed variables were $\log_e$-transformed and C5a, sC5b-9, cIMT and AAIx were converted to their standardised (Z-) scores. Student's t- or Mann–Whitney U-tests were used to compare continuous variables, and Pearson Chi^2 to compare categorical variables. We performed linear and logistic regression analyses with adjustments for age, sex, glucose metabolism status, body mass index, waist, lipids, blood pressure, renal function, physical activity, smoking and use of medication.

Table 1 shows characteristics of the study population as a whole and according to median plasma C5a and sC5b-9 concentrations. Participants with higher C5a or sC5b-9 levels were more often female, but did not show relevant differences in age, blood pressure, blood lipids or use of medication. Only CRP was higher in individuals above the median of C5a and/or sC5b-9, but there were no differences in cIMT, AAIx or prevalent CVD.

In linear regression analyses (Table 2), C5a and sC5b-9 were positively associated with the low-grade inflammation score. In the fully adjusted model, C5a and sC5b-9 were, per one SD increase, associated with 0.180 SD [0.105; 0.255] and 0.217 SD [0.143; 0.292] higher inflammation score, respectively. Also, most associations of C5a and sC5b-9 with the individual inflammatory markers were positive and significant (Fig. 1). Upon exclusion of subjects with CRP concentrations indicative of more acute inflammation (CRP > 10 mg/ml), these associations remained highly significant (data not shown). Furthermore, C5a and sC5b-9 were positively associated with the endothelial dysfunction score (fully adjusted model, β(C5a) = 0.080 SD [95%CI: 0.003; 0.156] and β(sC5b-9) = 0.127 SD [0.051; 0.203]). In the analysis of the individual endothelial dysfunction markers (Fig. 2), C5a was positively associated only with vWF, while sC5b-9 was positively associated with vWF, sEselectin and sVCAM-1. In contrast, C5a and sC5b-9 were not associated with cIMT or AAIx (Table 2). Also, in logistic regression analyses C5a and sC5b-9 were not associated with prevalent CVD (Table 2, fully adjusted model, OR(C5a) = 0.92 [0.75, 1.14] and OR(sC5b-9) = 0.98 [0.79, 1.21]).

The present findings indicate that, in humans, C5a and sC5b-9 may not only play a role in acute but also in chronic, low-grade inflammation. Furthermore, our findings suggest that C5a and sC5-9 may participate in endothelial dysfunction, which extends previous in-vitro studies that showed C5a to release vWF from endothelial cells [13] and (s)C5b-9 to induce different markers of endothelial activation [14,15]. However, C5a and sC5b-9 were not associated with markers of atherosclerosis cIMT or AAIx. Previously, C5a and sC5b-9 have been implicated in atherosclerosis in animal and in-vitro studies, and were also detected in human

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Table 1
Characteristics of the total study population and according to median plasma C5a and median plasma sC5b-9 concentrations.

	Study population	According to C5a concentration			According to sC5b-9 concentration		
	N = 537	<Median (1.2–7.2 ng/ml) N = 268	>Median (7.2–20.5 ng/ml) N = 269	P-value	<Median (32–109 ng/ml) N = 268	>Median (109–272 ng/ml) N = 269	P-value
Age (years; mean ± SD)	59.4 ± 6.9	59.5 ± 6.8	59.3 ± 7.1	0.77	59.5 ± 6.9	59.3 ± 7.0	0.76
Sex (male)	61	67	55	<0.01	67	55	<0.01
BMI (kg/m^2)	28.6 ± 4.4	28.5 ± 4.2	28.7 ± 4.6	0.63	28.3 ± 4.1	29.9 ± 4.7	0.18
Systolic blood pressure (mmHg)	140 ± 19	141 ± 19	139 ± 19	0.23	139 ± 18	141 ± 19	0.49
Total cholesterol (mmol/l)	5.2 ± 1.0	5.3 ± 1.0	5.2 ± 0.9	0.09	5.2 ± 1.0	5.3 ± 0.9	0.51
Triacylglycerides (mmol/L)	1.40 [1.00–2.00]	1.40 [1.00–1.90]	1.40 [1.00–2.00]	0.97	1.40 [1.00–2.00]	1.40 [1.00–1.90]	0.27
CRP (mg/L)	2.04 [0.94–3.97]	1.67 [0.86–3.19]	2.51 [1.08–5.02]	<0.01	1.70 [0.81–3.34]	2.47 [1.10–4.63]	<0.01
Current smokers/Ex-smokers (%)	21/50	20/50	23/51	0.57	23/48	19/53	0.40
Use of anti-hypertensive medication (%)	39	35	42	0.10	36	41	0.20
Use of lipid-lowering medication (%)	20	19	20	0.93	19	20	0.60
Use of glucose-lowering medication (%)	13	12	15	0.21	15	12	0.44
NGM/IGM/T2DM (%)	51/23/26	56/19/25	47/26/27	0.10	51/23/26	51/23/26	0.99
cIMT (mm)[a]	0.78 ± 0.16	0.78 ± 0.16	0.77 ± 0.16	0.31	0.79 ± 0.16	0.77 ± 0.15	0.17
AAIx[b]	1.09 ± 0.13	1.10 ± 0.14	1.09 ± 0.12	0.38	1.10 ± 0.12	1.09 ± 0.14	0.43
Cardiovascular disease (%)	28	28	28	0.98	29	28	0.83

Normally distributed variables are presented as mean ± standard deviation (SD). Skewed variables are presented as medians with interquartile range.
AAIx indicates ankle−arm blood pressure index; BMI, body mass index; cIMT, carotid–intima media thickness; CRP, C-reactive protein; IGM, impaired glucose metabolism; NGM, normal glucose metabolism; T2DM, type 2 diabetes.
Data are presented as mean ± standard deviation (normally distributed variables), median [interquartile range] (skewed variables) or proportion (%, categorical variables). P-values were obtained by ANOVA, Mann–Whitney *U* test or Pearson chi^2.
[a] cIMT was analysed in 496 participants.
[b] AAIx was analysed in 533 participants.

plaque material [16–18]. Our findings do not preclude that C5a and sC5b-9 play a role at the sites of atherosclerosis; rather they indicate that systemic concentrations do not appear to be related to atherosclerosis. C5a and sC5b-9 were also not associated with prevalent CVD. Previous studies that included hospital patients with acute or advanced CVD showed C5a and sC5b-9 to predict adverse outcomes. The lack of an association with prevalent CVD in our study suggests that, in individuals with stable CVD from the general population, systemic activation of the terminal complement pathway seems to be less prominent. As one major limitation of our study is its cross-sectional design, we cannot draw conclusions on the causality of the presented relationships.

In conclusion, systemic activation of the terminal complement pathway may play a role in chronic inflammation and in endothelial dysfunction. However, plasma concentrations of C5a and sC5b-9 were not associated with markers of atherosclerosis or with prevalent CVD. Compared to their demonstrated role in acute cardiovascular events, systemic increases in C5a and sC5b-9 appear less prominent in stable atherosclerosis and in patients with established CVD from the general population.

Table 2
Associations of C5a and sC5b-9 with low-grade inflammation, endothelial dysfunction, markers of atherosclerosis and prevalent CVD.

	Effect per 1 SD increase C5a		Effect per 1 SD increase sC5b-9	
	β [95% CI]	P-value	β [95% CI]	P-value
Association with the inflammatory score (in SD, N = 537)				
Model 1	0.205 [0.125; 0.285]	<0.001	0.238 [0.159; 0.317]	<0.001
Model 2	0.180 [0.105; 0.255]	<0.001	0.217 [0.143; 0.292]	<0.001
Association with the endothelial dysfunction score (in SD, N = 530)				
Model 1	0.105 [0.025, 0.185]	0.010	0.136 [0.056; 0.216]	0.001
Model 2	0.080 [0.003; 0.156]	0.042	0.127 [0.051; 0.203]	0.001
Association with cIMT (in SD, N = 496)				
Model 1	−0.006 [−0.092; 0.080]	0.886	−0.029 [−0.114; 0.055]	0.495
Model 2	−0.006 [−0.090; 0.078]	0.889	−0.059 [−0.142; 0.023]	0.159
Association with AAIx (in SD, N = 533)				
Model 1	0.033 [−0.048; 0.115]	0.418	0.014 [−0.066; 0.095]	0.725
Model 2	0.041 [−0.038; 0.120]	0.307	0.024 [−0.054; 0.103]	0.542
	OR [95% CI]	P-value	OR [95% CI]	P-value
Association with prevalent CVD (N = 537)				
Model 1	1.01 [0.82; 1.23]	0.950	0.99 [0.82; 1.21]	0.944
Model 2	0.92 [0.75; 1.14]	0.465	0.98 [0.79; 1.21]	0.816

Model 1: adjusted for age [years]; sex [men/women]; impaired glucose metabolism [yes/no]; type 2 diabetes [yes/no].
Model 2: Model 1 + body mass index [kg·(m^2)$^{-1}$]; waist [cm]; triacylglycerides [mmol/L]; total cholesterol [mmol/L]; high-density lipoprotein cholesterol [mmol/L]; systolic blood pressure [mm Hg]; eGFR [ml·1.73 m^2·(min)$^{-1}$]; smoking [packyears]; physical activity [total score]; use of glucose-lowering medication [yes/no]; use of anti-hypertensive medication [yes/no].
Skewed variables were $\log_e$ transformed prior to the analyses.