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Original Scientific Paper

Complement C3 and C4 in plasma and incidence of myocardial infarction and stroke: a population-based cohort study

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Background **Complement factor C3 and C4 have been associated with atherosclerosis and cardiovascular risk factors. This study explored whether plasma levels of C3 and C4 are risk factors for the incidence of cardiovascular disease (CVD).**
Design **A population-based prospective study of 5850 initially healthy men, 28–61 years old at baseline.**
Methods **Plasma levels of C3 and C4 were analysed at the baseline examination. The incidence of coronary events (i.e. fatal or non-fatal myocardial infarction), ischaemic stroke and cardiovascular events (i.e. myocardial infarction, ischaemic stroke or cardiovascular death) was studied over 18 years of follow-up.**
Results **Adjusted for age, C3 in the fourth quartile (versus the first quartile) was associated with an increased incidence of coronary events [relative risk (RR) 1.54, 95% confidence interval (CI) 1.2–1.9], cardiovascular events (RR 1.56, 95% CI 1.3–1.9), and non-significantly with the incidence of ischaemic stroke (RR 1.31, 95% CI 0.89–1.8). However, after adjustments for smoking, body mass index (BMI), cholesterol, diabetes and systolic blood pressure, these relationships were completely attenuated and non-significant. The relationships were similar for C4 concentrations within the normal range. However, for men with C4 in the top 10% of the distribution (>0.34 g/l), a significantly increased incidence of coronary events was found, which persisted after adjustments for risk factors.**
Conclusion **C3 and C4 show substantial correlations with cardiovascular risk factors, including blood pressure, BMI, and lipids. This relationship accounts for the increased incidence of CVD in men with high C3 levels. However, very high C4 levels may be associated with the incidence of CVD, independently of traditional cardiovascular risk factors.** ***Eur J Cardiovasc Prev Rehabil*** **14:392–397 © 2007 The European Society of Cardiology**

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Keywords: complement, C3, C4, epidemiology, inflammation, myocardial infarction, stroke

Introduction

Complement factor C3 and C4 are acute phase proteins and important components of the complement pathways of the immune system [1,2]. Even though hepatic production is the main source of C3 and C4, many cell types, including adipose tissue and vascular cells, express these proteins [3,4]. Inflammatory processes play a key role in atherogenesis [5], and many studies suggest that complement activation is involved in this process [6–13]. C3, C4 and other complement components are retained in the atherosclerotic lesions, and the complement system is activated in the atherosclerotic plaque [7,9,11]. Studies of C3-deficient mice suggest that an intact complement system is required for the development of the late stages of atherosclerosis [8].

Studies of human populations have shown that C3 and C4 are associated with increased levels of cardiovascular risk factors, for example, obesity, hypertension, lipids and diabetes [3,14–17]. Longitudinal studies have shown that C3 is a risk factor for developing diabetes and weight gain [16,18]. However, few studies have explored the relationship between plasma levels of C3 or C4 and cardiovascular disease (CVD). Case–control studies of individuals with a history of CVD have shown raised C3 [14,15,19,20] or C4 [21] concentrations compared with healthy controls. To our knowledge, there is only one prospective study of C3 and the incidence of CVD [22]. That study reported an

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increased age-adjusted incidence of CVD in men with high C3 levels, but it is unclear whether this relationship persisted after adjustments for other risk factors.

The purpose of the present study was to explore the relationships between C3 and C4 and the incidence of myocardial infarction and stroke.

Subjects and methods

Between 1974 and 1984, 22 444 men participated in a screening programme for the detection of individuals with a high risk of CVD. Complete birth cohorts from the city of Malmö were invited [23]. The participation rate was 71%. A determination of plasma proteins was part of the programme for 6193 men, selected at random from cohorts examined between 1974 and 1982. After the exclusion of men with a history of myocardial infarction, stroke or cancer (according to a questionnaire), 6075 men remained. Of them, information about C3 and C4 was available for 5859 and 5850 men, respectively.

The health service authority of Malmö approved and funded the screening programme. All participants gave informed consent.

Baseline examinations

Weight and height were measured in the morning with the subject wearing light indoor clothing and no shoes. The body mass index (BMI) was calculated as weight/height squared (kg/m^2).

Blood pressure (mmHg) was measured twice in the right arm after a 10-min rest. The average of two measurements was used. A sphygmomanometer and a rubber cuff of appropriate size were used. The use of antihypertensive medication was assessed in a questionnaire.

Subjects were categorized into smokers and non-smokers. Alcohol consumption was assessed by means of the modified shortened version of the Michigan Alcoholism Screening Test [24]. Men with more than two (out of nine) affirmative answers were considered to be high consumers of alcohol.

Physical inactivity was assessed in a questionnaire. Men who reported that they were mostly sedentary in their spare time were categorized as physically inactive. Subjects who confirmed a doctor's diagnosis of angina pectoris or who used nitrates were considered to have angina pectoris. Recent respiratory infection was recorded if it had occurred within 3 weeks before the examination.

The occupational level was categorized into low (manual occupation, low-level non-manual occupation) and high (medium or high-level non-manual occupation, entrepreneurs) occupational level. Men with other occupations or early retired men ($n = 150$) were categorized as having a low occupational level [25].

Blood samples were taken after an overnight fast. Serum cholesterol was analysed using standard methods at the laboratory of the university hospital. Men with fasting whole blood glucose of 6.1 mmol/l or greater, men with 2-h glucose values if 10.0 mmol/l or greater (glucose load 30 g/m^2 body surface area) and men who reported treatment for diabetes were considered to have diabetes.

An electroimmunoassay was used to assess the concentrations of plasma proteins [26]. The coefficient of variation was less than 5% [26]. The analysis was performed consecutively at study entry. C3 and C4 were originally expressed as the percentages of the mean values from a reference population of blood donors. The reference values for C3 were 70–130%, which corresponds to 0.67–1.29 g/l, and the reference values for C4 were 65–170% (0.13–0.32 g/l). In order to facilitate the interpretation of the C3 and C4 values, the percentages have been converted into grams/litre (C3 100% = 0.98 g/l, C4 100% = 0.20 g/l).

Follow-up

The procedures for case retrieval have been described previously [27]. In short, the Swedish Hospital Discharge Register, the Stroke Register of Malmö and the Swedish Cause of Death Register were used. A coronary event was defined as a first non-fatal myocardial infarction (code 410 according to the International Classification of Diseases, ICD, 9th revision) or death as a result of ischaemic heart disease (i.e. ICD codes 410–414). Cases with ICD codes 434 (cerebral infarction, $n = 162$) or 436 (unspecified stroke, $n = 30$) were considered to have ischaemic stroke. A cardiovascular event was defined as non-fatal ischaemic stroke, non-fatal myocardial infarction or death from CVD (ICD-9 codes 390–448), whichever came first. All men were followed from the baseline examination until death, emigration from Sweden or 31 December 1997 (mean follow-up 18.2 ± 4.2 years).

Statistics

One-way analysis of variance and the Mantel–Haenzel chi-squared test were used to compare the risk factors by quartiles of C3 and C4. Pearson's correlation was used for two continuous variables. Backward multiple linear regressions (P for remove > 0.10), with C3 or C4 as dependent variables, were used to study the multivariate relationships with other cardiovascular risk factors.

Cox regression was used to study the incidence of CVD by quartile of C3 and C4, with adjustments for confounding factors. P values for trends were obtained by modelling the quartiles as ordinal variables. The relationships with the incidence of CVD were also explored using C3 and C4 as continuous variables

(per 1 standard deviation). The findings from these analyses led us to explore whether very high C3 or C4 levels were associated with increased risk. C3 and C4 concentrations above the 90th percentile were considered to be very high. Two-tailed *P* values less than 0.05 were considered statistically significant. SPSS software (version 11.5; SPSS Inc., Chicago, Illinois, USA) was used for the statistical calculations.

Results

Relationships between C3, C4 and cardiovascular risk factors at baseline

Tables 1 and 2 present the relationships between quartiles of C3 and C4 and cardiovascular risk factors. C3 was positively and significantly associated with blood pressure, BMI, cholesterol, triglycerides and the prevalence of diabetes. C3 was not associated with age or smoking.

C4 was positively and significantly associated with age, blood pressure, BMI, cholesterol, triglycerides, and the prevalence of smoking and physical inactivity (Table 2). The correlation between C3 and C4 was $r = 0.50$ in this cohort ($P < 0.0001$).

All risk factors in Tables 1 and 2 were entered into a backward multiple regression model, with C3 or C4 as dependent variables, in order to explore the independent relationships with C3 and C4, respectively. Age ($\beta = -0.002$, $P = 0.02$), BMI ($\beta = 0.017$, $P < 0.001$), recent respiratory infection ($\beta = 0.033$, $P < 0.001$), diastolic blood pressure ($\beta = 0.001$, $P < 0.001$), cholesterol ($\beta = 0.014$, $P < 0.001$), log triglycerides ($\beta = 0.065$, $P < 0.001$), physical inactivity ($\beta = 0.015$, $P = 0.003$), diabetes ($\beta = 0.022$, $P = 0.07$), and low occupational level ($\beta = 0.016$, $P = 0.005$) were associated with C3 in the final model. Variables associated with C4 in the final model were BMI ($\beta = 0.002$, $P < 0.001$), diabetes ($\beta = -0.01$, $P = 0.02$), systolic blood pressure ($\beta = 0.0002$, $P < 0.001$), cholesterol ($\beta = 0.006$, $P < 0.001$), log triglycerides ($\beta = 0.014$, $P < 0.001$), smoking ($\beta = 0.013$, $P < 0.001$), recent respiratory infection ($\beta = 0.008$, $P = 0.03$) and low occupational level ($\beta = 0.005$, $P = 0.03$).

C3 and incidence of cardiovascular disease

The mean C3 concentrations at baseline were 1.05 ± 0.23 g/l in men who subsequently suffered a coronary event ($n = 590$) and 1.01 ± 0.22 g/l in men without coronary events during the follow-up ($n = 5269$; $P < 0.0001$).

Table 3 presents the incidence of CVD in relation to quartiles of C3. After adjustment for age, C3 in the top quartile was associated with a significantly increased incidence of coronary events [relative risk (RR) 1.54, $P < 0.0001$], and cardiovascular events (RR 1.56, $P < 0.0001$). Although not significantly, the age-adjusted incidence of ischaemic stroke (RR 1.31, $P = 0.17$) was also increased. However, after adjustment for smoking, BMI, cholesterol, diabetes, systolic blood pressure, and blood pressure medication, these relationships were completely attenuated and non-significant. The results were essentially the same if C3 was used as a continuous variable (per 1 SD increase; not shown).

C3 levels in the 90th percentile (≥ 1.30 versus < 1.30 g/l) were similarly associated with the incidence of coronary events (Table 3). However, after adjustment for risk factors, there was no relationship between C3 in the 90th percentile and the incidence of CVD (Table 3).

The relationships were virtually identical after further adjustments for occupation, alcohol consumption, physical inactivity, log triglycerides, recent respiratory infections or angina (not shown).

Table 1 **Cardiovascular risk factors in relation to quartiles of C3**

	Quartiles of C3				*P* (trend)
	Q1	Q2	Q3	Q4	
g/l	0.34–0.86	0.87–0.98	0.99–1.15	1.16–2.19	
N	1525	1571	1319	1444	
Age (years)	46.8 ± 3.8	46.9 ± 3.7	46.9 ± 3.9	46.8 ± 3.6	0.88
Smokers (%)	48	50	46	48	0.76
Systolic BP (mmHg)	126 ± 15	128 ± 15	130 ± 16	132 ± 17	<0.0001
Diastolic BP (mmHg)	85 ± 9.4	87 ± 9.6	88 ± 10	89 ± 11	<0.0001
BP treatment (%)	3.1	3.6	5.8	6.5	<0.0001
BMI (kg/m^2)	23.6 ± 2.8	24.6 ± 3.0	25.4 ± 3.2	26.4 ± 3.6	<0.0001
Cholesterol (mmol/l)	5.5 ± 1.0	5.7 ± 1.0	5.8 ± 1.0	5.9 ± 1.1	<0.0001
Triglycerides (mmol/l)*	1.17	1.31	1.44	1.66	<0.0001
Diabetes (%)	3.6	4.9	4.8	8.1	<0.0001
Angina (%)	1.2	1.0	1.1	1.9	0.13
Physical inactivity (%)	54	54	56	62	<0.0001
High alcohol consumption (%)	13	12	14	15	0.07
Low occupation (%)	60	60	62	67	<0.001
Recent respiratory infection (%)	8.6	7.7	9.3	11.8	0.001

BMI, Body mass index; BP, blood pressure. *Presented as geometric means.

Table 2 Cardiovascular risk factors in relation to quartiles of C4

	Quartiles of C4				*P* (trend)
	Q1	Q2	Q3	Q4	
g/l	0–0.18	0.19–0.23	0.24–0.28	0.29–0.68	
N	1489	1450	1451	1460	
Age (years)	46.7 ± 3.9	46.9 ± 3.9	46.7 ± 3.5	47.0 ± 3.8	0.04
Smokers (%)	41	48	50	54	<0.0001
Systolic BP (mmHg)	127 ± 15	129 ± 15	130 ± 16	130 ± 16	<0.0001
Diastolic BP (mmHg)	86 ± 9.3	87 ± 9.9	88 ± 10	88 ± 11	<0.0001
BP treatment (%)	3.1	5.8	4.6	5.1	0.04
BMI (kg/m^2)	24.5 ± 3.1	24.9 ± 3.3	25.2 ± 3.3	25.4 ± 3.3	<0.0001
Cholesterol (mmol/l)	5.5 ± 1.1	5.7 ± 1.0	5.7 ± 1.0	5.9 ± 1.0	<0.0001
Triglycerides (mmol/l)*	1.24	1.35	1.42	1.51	<0.0001
Diabetes (%)	4.9	5.6	6.1	4.7	0.92
Angina (%)	0.9	1.5	1.2	1.6	0.22
Physical inactivity (%)	55	56	57	58	0.03
High alcohol consumption (%)	12	13	14	15	0.005
Low occupation (%)	60	59	63	66	<0.001
Recent respiratory infection (%)	8.0	9.3	8.5	11.4	0.006

BMI, Body mass index; BP, blood pressure. *Presented as geometric means.

Table 3 Incidence of coronary events and stroke in relation to C3

	Quartiles of C3				90th percentile[a]
	Q1	Q2	Q3	Q4	
g/l	0.34–0.86	0.87–0.98	0.99–1.15	1.16–2.19	≥ 1.30 g/l
N	1525	1571	1319	1444	576
Coronary events, *n*	131	160	123	176	74
Age-adjusted RR (CI)	1.00	1.18 (0.9–1.5)	1.12 (0.9–1.4)	1.54 (1.2–1.9)	1.43 (1.12–1.8)
Risk-factor adjusted RR (CI)	1.00	1.02 (0.8–1.3)	0.87 (0.7–1.1)	1.05 (0.8–1.3)	1.05 (0.82–1.4)
Ischaemic stroke, *n*	50	45	41	56	21
Age-adjusted RR (CI)	1.00	0.87 (0.6–1.3)	0.99 (0.7–1.5)	1.31 (0.89–1.9)	1.23 (0.78–1.9)
Risk-factor adjusted RR (CI)	1.00	0.77 (0.5–1.2)	0.77 (0.5–1.2)	0.90 (0.6–1.4)	0.92 (0.6–1.5)
Cardiovascular events, *n*	185	206	173	248	99
Age-adjusted RR (CI)	1.00	1.08 (0.9–1.3)	1.12 (0.9–1.4)	1.56 (1.3–1.9)	1.39 (1.13–1.7)
Risk-factor adjusted RR (CI)	1.00	0.94 (0.8–1.1)	0.89 (0.7–1.1)	1.08 (0.9–1.3)	1.03 (0.83–1.3)

CI, 95% Confidence interval; RR, relative risk. [a]Compared with men with C3 below the 90th percentile (<1.30 g/l). Risk factors: age, smoking, body mass index, cholesterol, diabetes, systolic blood pressure, blood pressure medication.

C4 and incidence of cardiovascular disease

The mean C4 concentrations at baseline were 0.26 ± 0.09 g/l in men who subsequently suffered a coronary event ($n = 589$) and 0.24 ± 0.08 g/l in men without coronary events during the follow-up ($n = 5261$; $P < 0.0001$).

Table 4 presents the incidence of CVD in relation to quartiles of C4. C4 in the top quartile was similarly associated with an increased incidence of coronary events (RR 1.50, $P = 0.004$) and cardiovascular events (RR 1.34, $P = 0.003$) after adjustments for age. These relationships were attenuated after adjustments for established risk factors, in accordance with the finding for C3. However, when C4 was used as a continuous variable (per 1 SD, 0.08 g/l), there was a significant relationship between C4 and the incidence of cardiac events, even after adjustments for risk factors [RR per 1 SD 1.09, 95% confidence interval (CI) 1.01–1.18]. It was found that this relationship was explained by an increased incidence among men with very high C4 levels (90th percentile, > 0.34 versus ≤ 0.34 g/l). C4 levels in the 90th percentile were associated with the incidence of coronary events and cardiovascular events even after adjustment for risk factors (Table 4). These relationships were largely unchanged after further adjustments for occupation, alcohol consumption, physical inactivity, log triglycerides, recent respiratory infections and angina (coronary events RR 1.36, 95% CI 1.08–1.72, cardiovascular events RR 1.30, 95% CI 1.06–1.59).

Discussion

Complement activation has been demonstrated in human atherosclerotic plaque and many studies suggest that complement activation could be involved in atherogenesis [6–13]. However, the relationships between C3, C4 and the incidence of CVD are unclear. This study shows that men with C3 or C4 in the top quartile have a significantly higher incidence of CVD. Traditional risk factors explained the relationship between C3 and the increased incidence of CVD. However, C4 levels above the 90th percentile were associated with the incidence of CVD, even after adjustment for risk factors.

Table 4 Incidence of coronary events and stroke in relation to quartiles of C4

	Quartiles of C4				90th percentile[a]
	Q1	Q2	Q3	Q4	
g/l	0–0.18	0.19–0.23	0.24–0.28	0.29–0.68	>0.34 g/l
N	1489	1450	1451	1460	575
Coronary events, *n*	123	136	148	182	88
Age-adjusted RR (CI)	1.00	1.11 (0.9–1.4)	1.22 (0.96–1.5)	1.50 (1.2–1.9)	1.68 (1.3–2.1)
Risk-factor adjusted RR (CI)	1.00	0.96 (0.8–1.2)	0.98 (0.8–1.2)	1.14 (0.9–1.4)	1.41 (1.12–1.8)
Ischaemic stroke, *n*	45	52	42	50	24
Age-adjusted RR (CI)	1.00	1.08 (0.7–1.6)	0.87 (0.6–1.3)	1.05 (0.7–1.6)	1.37 (0.89–2.1)
Risk-factor adjusted RR (CI)	1.00	0.98 (0.7–1.4)	0.72 (0.5–1.1)	0.85 (0.6–1.3)	1.21 (0.78–1.9)
Cardiovascular events, *n*	181	192	200	238	113
Age-adjusted RR (CI)	1.00	1.06 (0.87–1.3)	1.11 (0.91–1.4)	1.34 (1.1–1.6)	1.57 (1.3–1.9)
Risk-factor adjusted RR (CI)	1.00	0.94 (0.8–1.15)	0.90 (0.7–1.1)	1.05 (0.86–1.3)	1.34 (1.09–1.6)

CI, 95% Confidence interval; RR, relative risk. [a]Compared with men with C4 below the 90th percentile (≤ 0.34 g/l). Risk factors: age, smoking, body mass index, cholesterol, diabetes, systolic blood pressure, blood pressure medication.

Muscari and coworkers [22] reported an increased incidence of myocardial infarction in men with C3 concentrations in the upper third, but no similar association in women. The results, which were based on 38 male and 19 female cases, were only adjusted for age and sex, and it is unclear whether they persisted after adjustment for risk factors. The authors did not find any relationship between C3 and stroke. C4 was unrelated to CVD in their study [22]. To our knowledge, this is the only previous prospective study of C3 or C4 and the incidence of CVD.

Raised C3 or C4 levels have been associated with CVD in cross-sectional studies and case–control studies [14,15,19–21]. The relationships have remained significant after adjustment for other risk factors. However, the covariates vary between the studies, and not all studies have adjusted for all important confounding factors. C3 was associated with a worse prognosis in a study of patients with severe coronary heart disease [19]. The results from studies of patients with established CVD are thus at variance with our results. Elevated C3 in plasma may be related to the atherosclerotic burden or to the myocardial injury, rather than the development of CVD in initially healthy subjects. It has been shown that complement is activated in atherosclerotic lesions [7–9] and in the infarcted myocardium [28], but there is less evidence for any significant complement activation in normal arteries. Furthermore, even though complement activation plays a role in atherogenesis, it is unclear whether this is reflected by plasma levels of C3 and C4.

C4 is involved in the classical pathway of complement activation. C-reactive protein (CRP) and immune complexes are important activators of the classical pathway [2]. CRP and complement components co-localize in atherosclerotic plaque, and it has been suggested that CRP could promote atherosclerosis through complement activation [10]. We observed an increased incidence of coronary events in men with C4 in the top 10% of the distribution. It is conceivable that high C4 levels are associated with increased complement activation in atherosclerotic plaque, which could increase the incidence of coronary events. It is also possible that systemic inflammation and proinflammatory molecules that stimulate the hepatic production of C4 are responsible for the increased incidence of coronary events. The relationships between high C4 and coronary events persisted after adjustment for other cardiovascular risk factors. However, further studies are needed to explore the role of plasma levels of C4 in the incidence of CVD.

A full understanding of the relationships between complement factors and CVD must encompass the reasons why C3 and C4 correlate with BMI, blood pressure, glucose and lipids. Previous studies from this cohort show that C3 is a risk factor for developing diabetes and weight gain [16,18]. The reasons for these associations are unclear. Acylation stimulating protein (ASP) is a peptide hormone, which is formed by the protolytic cleavage of C3. ASP has several metabolic effects; for example it stimulates the uptake of glucose and fatty acids in adipose tissue [29]. A blunted response to the C3–ASP system could hypothetically be related to increased levels of C3, glucose and lipids [30]. Another possible explanation could be that cytokines that stimulate the hepatic production of C3 could also stimulate the production of lipids and reduce insulin sensitivity. Furthermore, because C3 and C4 is expressed and produced in abdominal adipose tissue [3], which is known to be metabolically active, this could also explain the relationship between C3, C4 and other metabolic risk factors.

The large numbers of individuals and events are a major strength of this study. The endpoints were retrieved from national and local hospital registers. These registers cover the southern parts of Sweden during the entire follow-up period. The Stroke Register of Malmö includes both hospitalized and non-hospitalized cases, and each stroke was validated by a review of the hospital records. A few subjects who moved away from Sweden were censored at

the time of emigration. This number was small and cannot influence the results.

The laboratory analyses were limited to those who were available in clinical practice at the time of screening. For example, we have no information about high-density lipoprotein cholesterol. However, according to the present results, the traditional cardiovascular risk factors were sufficient to account for the relationship between C3 and CVD. We cannot rule out the possibility that an unmeasured risk factor could explain the relationships between C4 and CVD though.

Another limitation is that we do not know about changes that occurred during the follow-up period, for example, smoking cessation or changing levels of C3 or C4. However, the concentrations in healthy individuals are quite stable over time [31]. The survival curves from the present cohort (not shown) confirmed that the cardiovascular risk associated with C3 and C4 remained even after many years of follow-up.

C3 and C4 show substantial correlations with cardiovascular risk factors, including blood pressure, BMI, and lipids. This relationship accounts for the increased incidence of CVD in men with high C3 levels. However, very high C4 levels may be associated with increased cardiovascular risk, independently of traditional cardiovascular risk factors.

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