Leukocyte subsets and abdominal aortic aneurysms detected by screening in men

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Abstract. Langenskiöld M, Smidfelt K, Nordanstig J, Bergström G, Tivesten Å (Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden). Leukocyte subsets and abdominal aortic aneurysms detected by screening in men. J Intern Med 2020; 288: 345–355.

Objective. In the present case–control study, we describe the associations between leukocyte subsets in blood and early, screening-detected AAA in men. An abdominal aortic aneurysm (AAA) may result in a life-threatening rupture of the aortic wall. The trigger for AAA formation remains unknown, but the vascular adventitia of advanced AAAs is infiltrated by various leukocytes, indicating that the pathogenesis may involve inflammation.

Methods. In Sweden, all 65-year-old men are invited to an ultrasound examination for detection of AAA. At the Gothenburg screening site, 16 256 men were examined in 2013–2017, 1.2% of whom had an AAA (diameter of the infrarenal aorta ≥30 mm). All men with AAA at screening as well as a randomized selection of AAA-free screened men were invited to participate in a case–control study.

Results. The median diameter of AAAs was 33 mm. Men with an AAA (n = 151) had a higher frequency of smoking, hypertension and statin use than controls (n = 224). Blood levels of neutrophils, lymphocytes, monocytes and basophils were higher in individuals with an AAA, but eosinophil count did not differ from controls. Odds ratios (95% confidence interval) for AAA were 8.6 (4.2–17.4), 3.5 (1.9–6.6) and 3.3 (1.8–6.3) for the highest versus lowest quartile of neutrophils, lymphocytes and monocytes, respectively. For neutrophils and lymphocytes, the association with AAA remained significant after adjustment for smoking and other known risk factors/markers.

Conclusion. Several, but not all, subsets of circulating leukocytes are associated with screening-detected AAA in men, which is insufficiently explained by associations with smoking and other confounders.

Keywords: abdominal aortic aneurysm, blood, leukocytes, men, screening.

Introduction

An abdominal aortic aneurysm (AAA) is a pathological widening of the abdominal aorta, which may result in a rupture of the aortic wall that is associated with a high mortality [1]. Male sex is a strong risk factor for AAA and ultrasound screening of men for AAA has been initiated in a few countries, including the United Kingdom and Sweden. Screening for AAA in men at 65 years of age is now nationwide in Sweden with a near 100% coverage of invitations [2].

In addition to male sex, risk factors for AAA include advancing age, smoking and family history of AAA [3]. Hypertension, hypercholesterolaemia and low-density lipoprotein (HDL) cholesterol levels also have been associated with the disease [3]. The trigger for AAA formation remains unknown, but there is accumulating evidence that the pathogenesis involves inflammation, proteolytic degradation and smooth muscle cell death in the arterial wall [4]. In advanced AAAs, the vascular adventitia is infiltrated by various leukocytes, including neutrophils, lymphocytes and monocytes/macrophages, and it has been proposed that these cell types may be involved in the pathogenesis of AAA [4].

In epidemiological studies, a role of inflammation in AAA is supported by increased cytokine levels, high-sensitivity C-reactive protein and other inflammation-sensitive proteins in AAA patients.
[5-8]. In a prospective population-based study, total white blood cell count was higher in participants that were hospitalized with a diagnosis of AAA [9]. In the population-based ARIC study, total white blood cell count was higher amongst participants that were hospitalized with a diagnosis of AAA and also in participants with asymptomatic ultrasound-detected AAA compared with AAA-free participants [6]. However, there are to date no epidemiological studies addressing the association between differential white blood cell counts and asymptomatic screening-detected AAA.

In the present study, we describe the associations between subsets of circulating leukocytes and presence of screening-detected AAA in 65-year-old men in a case–control study based on ultrasound screening of more than 16 000 Swedish men.

**Materials and methods**

**Study population**

In the nationwide abdominal aortic aneurysm screening programme in Sweden, all men aged 65 years consecutively identified through the National Population Register (which is updated every third month) are invited to an ultrasound examination of the infrarenal aorta, regardless of any known AAA [2]. The aortic aneurysm screening programme was first initiated in Uppsala county in 2006 [10], and started in western Sweden (Region Västra Götaland) in 2008. The programme reached nationwide coverage in 2014. The early experiences and outcomes of the national aortic screening programme (2006–2014) have previously been reported [2]. In western Sweden, the examination fee for the AAA screening visit was €10 during 2013–2017 and travel expenses were not reimbursed. Invitations are sent by regular mail, and a second invitation is sent out to those who do not respond to the first invitation. The screening visit examination consists of a single ultrasound scan where the maximum infrarenal anteroposterior diameter is measured according to the leading edge-to-leading edge method, with the ultrasound transducer placed longitudinally to the aorta. An AAA is defined as an aortic diameter ≥30 mm [11]. Individuals shown to have an AAA are contacted by telephone within two working days for follow-up information by a vascular surgeon.

In western Sweden (1.7 million inhabitants, approximately 1/6 of the Swedish population), subjects with an AAA are invited to participate in a case–control study called Gothia 3A. The inclusion criterion of the study is an AAA diagnosed during the population-based screening examination, and the exclusion criterion is the inability to understand written Swedish study information or other reasons for not comprehending the study information. The study was approved by the Regional Ethical Review Board in Gothenburg, Sweden. All subjects gave written informed consent. Individuals with AAA receive a study invitation at their first outpatient clinic visit, within 4 weeks of the initial screening examination. AAA-free screened controls are recruited using a randomized consecutive enrolment. AAA-free controls receive a study invitation directly at the screening site when the absence of an AAA is established by ultrasound examination. A separate study visit for individuals with AAA and controls in the Gothia 3A study takes place within 3 months of the first screening examination, when blood sampling and questionnaire data are collected and clinical examinations performed.

The study population included in the present analysis consists of Gothia 3A subjects recruited from the Gothenburg screening area, comprising about half of the total screening population in western Sweden, between March 2013 and August 2017. The start of the study period was determined by the inclusion of blood sampling for differential white blood cell count in the local Gothia 3A study protocol.

**Differential white blood cell count**

Venous blood samples were drawn in the morning after an overnight fast. For analysis of a differential white blood cell count, blood was collected in K2-EDTA microtubes. Samples were analysed within 24 h by flow cytometry (Advia 2120i, Siemens Healthineers) at the accredited central laboratory at the Sahlgrenska University Hospital in Gothenburg. Reference ranges of the assay were neutrophils 1.8–7.5, lymphocytes 0.8–4.5, monocytes 0.1–1.0, cosinophils 0.04–0.4 and basophils 0–0.1 × 10⁹/L.

**Assessment of covariates**

A questionnaire was used to gather information about smoking habits, general health, medications, height and weight. Diabetes was defined as a self-reported medical diagnosis of diabetes. Prevalent cardiovascular disease was defined as a
self-reported medical diagnosis of myocardial infarction, angina pectoris or heart failure. Prevalent cerebrovascular disease was defined as a self-reported medical diagnosis of stroke or transient ischaemic attack. Prevalent asthma/chronic obstructive pulmonary disease (COPD) was defined as self-reported asthma, chronic bronchitis or COPD. A supine systolic blood pressure was measured following a 10 min period of rest. Serum total and HDL cholesterol were determined by fully enzymatic techniques and photometry according to standardized in-house methodology (Cobas 8000, Roche Diagnostica Scandinavia AB) at the accredited central laboratory at the Sahlgrenska University Hospital in Gothenburg.

**Statistical methods**

The frequencies of smoking, use of snuff, diabetes, use of anti-hypertensive medication and use of statins between cases and controls were tested by the chi-square test. For continuous variables (weight, height, systolic blood pressure, total cholesterol and HDL cholesterol), the corresponding analyses were performed by t-test, but pack-years and cigarettes/day amongst current smokers showed a skewed distribution and were analysed by Mann–Whitney U test.

Levels of leukocyte subsets were continuous data except for basophil count that only had two values and therefore was treated as a dichotomous variable. Differences in log-transformed leukocyte levels between cases and controls were analysed by t-test. Chi-square test was used for the corresponding analysis of basophil count. For descriptive statistics of leukocyte subsets, the geometric mean (antilog of mean of log-transformed values) and frequencies were presented, respectively. A permutation-based linear regression analysis of log-transformed leukocyte levels, with predictors group, smoking status and their interaction, was used to establish the significance of the interaction effect between smoking status and group (case/control); for each analysis, the P-value was based on 10 000 permutations.

Crude odds ratios (95% confidence intervals) for AAA were calculated by logistic regression for categories of leukocyte subsets. Neutrophils, lymphocytes, monocytes and eosinophils were studied across quartiles, whilst basophils were studied as a dichotomous variable. Leukocyte quartile was further included as a quadratic term in the logistic regression analyses to test for a possible nonlinear association between leukocyte quartile and AAA prevalence, yielding a P value < 0.05 for lymphocytes and eosinophils. Based on the observed estimates across quartiles and the results of the quadratic models, lymphocyte count was further examined as a dichotomous variable comparing quartile 4 with quartiles 1 to 3, eosinophils were analysed comparing quartile 1 versus quartile 2 to 4, whilst neutrophils and monocytes were analysed per quartile increase in a logistic regression model. In addition to a crude model, estimates were adjusted for current smoking (yes/no), pack-years (entered as quartiles because of a non-normal distribution of data), weight, height, systolic blood pressure, total cholesterol, HDL cholesterol, diabetes mellitus (yes/no), use of anti-hypertensive medications (yes/no), use of statins (yes/no), prevalent asthma/COPD (yes/no), prevalent cardiovascular or cerebrovascular diseases (yes/no). We further used 10-fold cross-validated logistic regression models to assess if leukocyte subsets were effective in predicting AAA.

We performed permutation-based linear regression and cross-validated logistic regression using R (version 3.5.1) with the stats and caret packages. For all other statistical analyses, we used SPSS for Windows (version 24.0.0.0; SPSS, Chicago, IL).

**Results**

In the Gothenburg screening area, screening invitations were sent to 20 114 men during the current study period (Fig. 1). Of these, 16 256 men (81%) attended the screening visit, 15 409 after the first invitation and 847 after the second invitation. Amongst the screened men, 191 had an AAA, 16 015 attended the screening visit, 15 409 after the first invitation and 847 after the second invitation. Amongst the screened men, 191 had an AAA, resulting in an AAA prevalence of 1.2%. Individuals with an AAA had an acceptance rate of 80% for participation in the Gothia 3A study. Amongst controls, 54% of those invited accepted participation (Fig. 1).

The median diameter of AAAs was 33 mm (range 30–98 mm; 25th–75th percentile 31–38 mm); 116/151 of the AAAs (77%) were small AAA (diameter of 30–39 mm).

The characteristics of men with an AAA (n = 151) and controls (n = 224) are shown in Table 1. As expected, those with an AAA were more likely to be current smokers and had a higher life-time
smoking burden (expressed as pack-years). Current smokers amongst AAA men tended to smoke more than current smokers amongst controls. Current use of snuff, moist powder tobacco for local oral application, was also more common amongst men with AAA. AAA further was associated with slightly higher body weight, increased systolic blood pressure and use of anti-hypertensive medication. Statin use was more common and serum total cholesterol was lower amongst men with AAA. The levels of HDL cholesterol were also lower, whilst the frequency of diabetes mellitus was similar between the groups and there was no significant difference in asthma/COPD. AAA was further associated with prevalent cardiovascular, but not cerebrovascular, disease (Table 1).

To address the potential association between leukocyte subsets and AAA, we first compared concentrations of the five different leukocyte subsets included in the differential white blood cell count between individuals with an AAA and controls. Those with an AAA had higher levels of neutrophils, lymphocytes, monocytes and basophils (Table 2). Eosinophil count did not differ significantly between the groups. The neutrophil-to-lymphocyte ratio was also higher in individuals with AAA (2.15 ± 0.84) compared to controls (1.97 ± 0.92; *P* = 0.028).

As smoking is the most important risk factor for AAA and also activates the immune system [12], we next studied leukocyte levels in current smokers versus nonsmokers amongst men with AAA and controls (Table 2). In AAA, current smoking was associated with higher levels of neutrophils and lymphocytes and a trend towards higher levels of monocytes and basophils. Amongst controls, current smoking was associated with higher levels of neutrophils, lymphocytes and monocytes with a
similar trend for basophils. There were no significant associations between leukocyte numbers and current use of snuff in either group. AAA presence remained associated with higher levels of neutrophils, lymphocytes and monocytes amongst current nonsmokers and nonusers of snuff, respectively (Table 2). Interactions between case–control group and smoking were evaluated by addition of interaction terms in permutation-based linear regression analyses. The tests showed a significant interaction between case–control group and smoking for lymphocyte count ($P = 0.017$), but not for neutrophils, monocytes or eosinophils. A box-and-whisker plot (Figure 2) illustrates that the difference in lymphocytes between current smokers versus nonsmokers was larger amongst cases than controls.

We next studied the association between AAA and categorized leukocyte subsets (Figure 3 and Table S1). Neutrophils, lymphocytes, monocytes and eosinophils were studied across quartiles, whilst basophils were studied as a dichotomous variable based on the distribution of data. Crude odds ratios for AAA were calculated for each quartile of neutrophils, lymphocytes, monocytes and eosinophils, with quartile 1 as reference. Analysing trend over quartiles, neutrophil, lymphocyte and monocyte quartiles all were associated with screening-detected AAA (Table S1). There was also a significant association between AAA and basophil category, whilst there was no significant association across eosinophil quartiles. For neutrophils and monocytes, the odds ratios for AAA tended to increase per quartile increase, whilst only the highest quartile of lymphocytes was associated with AAA presence (Fig. 3).

We next described crude and multivariate odds ratios for AAA in categories of leukocyte counts (Table 3) that were chosen based on the results of the quartile data and the result that including a quadratic term revealed a significant nonlinear association between AAA prevalence and lymphocyte and eosinophil quartiles, but not neutrophil or monocyte quartiles. For neutrophils, the increased

### Table 1 Characteristics of the study population

| AAA cases | Controls | $P$ Value |
|-----------|----------|-----------|
| n = 151   | n = 224  |           |
| Weight, kg| 90.1 ± 16.2 | 86.6 ± 14.0 | 0.032 |
| Height, cm| 179.3 ± 6.1  | 178.7 ± 6.3  | 0.39  |
| Smoking, n (%) |        |           |
| Never     | 16 (11.1) | 88 (39.3) | <0.001 |
| Past      | 86 (59.7) | 119 (53.1) | 0.21  |
| Current   | 42 (29.2) | 17 (7.6)  | <0.001 |
| Pack-years, n | 27.4 ± 20.5 | 9.2 ± 14.2 | <0.001 |
| Cigarettes/day amongst current smokers, n | 14.8 ± 7.9 | 11.0 ± 5.8 | 0.094 |
| Current use of snuff, n (%) | 39 (26.9) | 31 (13.9) | 0.002 |
| Systolic blood pressure, mmHg | 149.2 ± 19.8 | 139.7 ± 16.4 | <0.001 |
| Serum total cholesterol, mmol L$^{-1}$ | 5.05 ± 1.16 | 5.46 ± 1.11 | <0.001 |
| Serum HDL cholesterol, mmol L$^{-1}$ | 1.26 ± 0.39 | 1.51 ± 0.39 | <0.001 |
| Diabetes mellitus, n (%) | 17 (11.6) | 23 (10.3) | 0.68 |
| Use of anti-hypertensive medications, n (%) | 85 (56.3) | 97 (43.3) | 0.014 |
| Use of statins, n (%) | 56 (37.1) | 58 (25.9) | 0.021 |
| Prevalent asthma/COPD, n (%) | 14 (9.8) | 15 (6.9) | 0.33 |
| Prevalent asthma/COPD in former/current smokers, n (%) | 13 (9.1) | 12 (5.6) | 0.20 |
| Prevalent cardiovascular disease, n (%) | 38 (26.2) | 15 (6.7) | <0.001 |
| Prevalent cerebrovascular disease, n (%) | 16 (11.3) | 16 (7.2) | 0.18 |

The population consisted of men, all 65 years old. Values are mean ± SD unless otherwise indicated. Data were analysed by chi-square test or t-test, except pack-years and cigarettes/day amongst current smokers that were analysed by Mann-Whitney U test. AAA indicates abdominal aortic aneurysm; COPD, chronic obstructive pulmonary disease.
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Table 2  Levels of circulating leukocyte subsets in AAA cases and controls

|                | AAA cases                  | Controls                  | P Value |
|----------------|----------------------------|----------------------------|---------|
| Neutrophils, $\times 10^9$/L | $4.25 \pm 1.28$            | $3.39 \pm 1.23$            | $<0.001$ |
| Lymphocytes, $\times 10^9$/L | $2.00 \pm 0.69$            | $1.72 \pm 0.56$            | $<0.001$ |
| Monocytes, $\times 10^9$/L   | $0.45 \pm 0.17$            | $0.39 \pm 0.12$            | $<0.001$ |
| Eosinophils, $\times 10^9$/L | $0.16 \pm 0.30$            | $0.17 \pm 0.12$            | 0.38     |
| Basophils $>0 \times 10^9$/L, n (%) | 55 (36)          | 51 (23)                    | 0.004    |

|                | Current smoking (n = 42) | Nonsmokers (n = 102) | P value | Current smoking (n = 17) | Nonsmokers (n = 207) | P value |
|----------------|-------------------------|----------------------|---------|--------------------------|----------------------|---------|
| Neutrophils, $\times 10^9$/L | $4.94 \pm 1.39$         | $3.98 \pm 1.14^*$   | $<0.001$ | $4.02 \pm 1.46$         | $3.34 \pm 1.20$       | 0.028   |
| Lymphocytes, $\times 10^9$/L | $2.21 \pm 0.80$         | $1.88 \pm 0.61^*$   | 0.007   | $1.99 \pm 0.76$         | $1.70 \pm 0.53$       | 0.034   |
| Monocytes, $\times 10^9$/L   | $0.48 \pm 0.18$         | $0.43 \pm 0.15^*$   | 0.063   | $0.46 \pm 0.12$         | $0.38 \pm 0.12$       | 0.027   |
| Eosinophils, $\times 10^9$/L | $0.18 \pm 0.18$         | $0.15 \pm 0.34$     | 0.23    | $0.19 \pm 0.12$         | $0.17 \pm 0.12$       | 0.40    |
| Basophils $>0 \times 10^9$/L, n (%) | 20 (48)          | 32 (31)               | 0.065   | 7 (41)                   | 44 (21)               | 0.060   |

|                | Current use of snuff (n = 39) | Nonusers (n = 106) | P value | Current use of snuff (n = 31) | Nonusers (n = 192) | P value |
|----------------|-------------------------------|-------------------|---------|-------------------------------|-------------------|---------|
| Neutrophils, $\times 10^9$/L | $4.35 \pm 1.23$         | $4.21 \pm 1.32^*$ | 0.58    | $3.74 \pm 1.06$         | $3.34 \pm 1.25$   | 0.079   |
| Lymphocytes, $\times 10^9$/L | $1.93 \pm 0.63$         | $1.98 \pm 0.71^*$ | 0.67    | $1.75 \pm 0.53$         | $1.72 \pm 0.57$   | 0.75    |
| Monocytes, $\times 10^9$/L   | $0.46 \pm 0.15$         | $0.44 \pm 0.17^*$ | 0.46    | $0.39 \pm 0.10$         | $0.39 \pm 0.12$   | 0.80    |
| Eosinophils, $\times 10^9$/L | $0.18 \pm 0.12$         | $0.15 \pm 0.35$   | 0.38    | $0.18 \pm 0.09$         | $0.17 \pm 0.13$   | 0.59    |
| Basophils $>0 \times 10^9$/L, n (%) | 18 (46)          | 34 (32)               | 0.12    | 7 (23)                   | 43 (22)             | 0.98    |

Values are geometric mean ± SD or n (%). P values from t-test of log-transformed leukocyte levels and chi-square test, respectively. Nonsmokers indicate no current smoking.

*P < 0.01 vs. controls that are nonsmokers or nonusers of snuff, respectively.

odds ratio for AAA per quartile increase remained significant after adjusting for smoking and other known risk factors and risk markers. Similarly, the increased AAA risk amongst subjects in quartile 4 versus quartiles 1-3 for lymphocytes remained statistically significant after multivariate adjustment. The increased risk of AAA per quartile increase of monocytes remained after adjustment for current smoking, but was abolished by adjustment for smoking burden. There was a statistically nonsignificant trend towards relatively higher risk in the lowest eosinophil quartile compared with the higher quartiles, and the point estimate was not materially different in the multivariate model. The association between basophil count and AAA was abolished by multivariate adjustment.

Given the known associations between AAA and other vascular diseases [4], we further adjusted the neutrophil-AAA and lymphocyte-AAA associations for prevalent cardiovascular and cerebrovascular diseases (Table 3). We also adjusted for prevalent asthma/COPD. These adjustments did not materially alter the leukocyte-AAA associations. Taken together, higher neutrophil and lymphocyte counts were associated with the presence of AAA independently of other risk factors/markers.

We identified cut-off values for the best prediction of AAA for neutrophils ($4.74 \times 10^9$/L), lymphocytes ($2.45 \times 10^9$/L) and the neutrophil-to-lymphocyte ratio (4.15). Using these cut-offs, the cross-validated predictive accuracy was 64.95% for neutrophils, 62.38% for lymphocytes and 58.80% for the neutrophil-to-lymphocyte ratio.

Discussion

The present case–control study, based on ultrasound screening of more than 16,000 Swedish 65-
year-old men, addressed the potential association between AAA and subclasses of leukocytes included in a differential white blood cell count. Individuals with a screening-detected AAA showed higher levels of neutrophils, lymphocytes, monocytes, and basophils, whilst eosinophil count did not differ between individuals with and without an AAA. For neutrophils and lymphocytes, the association with screening-detected AAA remained after adjustment for smoking and other known risk factors and cardiovascular comorbidity.

To the best of our knowledge, the present study is the first to report associations between leukocyte subsets and asymptomatic screening-detected AAA. A higher total white blood cell count has been associated with incident hospitalization for AAA in two population-based cohort studies [6,9]. Further, a higher total white blood cell count was associated with future ultrasound detection of asymptomatic AAA in 74 men and women in the population-based ARIC study [6]. Our data show that several, but not all, subsets of circulating leukocytes are associated with screening-detected AAA in men.

Accumulating evidence suggest that poorly regulated inflammation contributes to aortic dilatation in AAA disease [8]. Different classes of leukocytes may be involved in and/or modulate the development of AAA, as suggested by their infiltration in human advanced aneurysms [8]. Our finding of an association between circulating levels of leukocyte subsets and screening-detected AAA may support the hypothesis of an early and broad involvement of the immune system in the pathogenesis of AAA [8,13]. Alternatively, AAA-associated factors, such as activated endothelium and thrombus formation may trigger leukocytosis, which may or may not be involved in disease development. In this association study, we cannot fully account for factors that may confound the leukocyte-AAA associations; however, the associations for neutrophils and lymphocytes remained in the models adjusting for established risk factors and cardiovascular comorbidity.

Neutrophils infiltrate AAA tissue, particularly intraluminal thrombi, which frequently are present in larger human AAA [8,14]. Results from the CALIBER study recently showed that high neutrophil counts are associated with clinical presentation of AAA, similar to other cardiovascular diseases [15]. Evidence also suggest that circulating markers of neutrophil activation are increased in AAA patients [4,8]. Our finding that higher circulating neutrophil counts are associated with screening-detected AAA may support the notion that neutrophils are associated with AAA also at earlier stages of disease development.

Both B and T cells infiltrate human AAA tissue [8] and here we found that high blood lymphocytes were associated with screening-detected AAA. No such association was found for clinically presented AAA in the CALIBER study [16]. Interestingly, the same study reported that low rather than high lymphocyte counts are associated with coronary heart disease [16]. Lymphocytes may be both protective and pathogenic, and an imbalance between pro-inflammatory and anti-inflammatory lymphocytes may determine disease progression [8]. In accordance, lower blood levels of regulatory T cells and higher levels of inflammatory Th17 cells have been observed in individuals with asymptomatic AAA [17].
Fig. 3  Crude odds ratios (95% confidence intervals) for screening-detected AAA in relation to categories of leukocyte subsets (a, neutrophils; b, lymphocyte; c, monocytes; d, eosinophils; e, basophils). For detailed description of categories/quartiles, see Table S1. Basophil category 1, 0 values; category 2, values > 0. Category/quartile 1 is used as reference.
The neutrophil-to-lymphocyte ratio is increasingly investigated as a marker of systemic inflammation and has been shown to predict outcomes after major cardio- and cerebrovascular events [18,19] as well as adverse outcomes after AAA rupture and elective abdominal aortic aneurysm repair [20]. In the present study, we found that the neutrophil-to-lymphocyte ratio was slightly higher in men with AAA, suggesting that this variable is associated with AAA also in an asymptomatic stage of the disease.

In accordance with evidence suggesting that smoking activates the immune system and may alter circulating leukocyte populations [12], we found that current smoking was associated with higher blood concentrations of neutrophils, lymphocytes and monocytes. By contrast, we found no significant associations between circulating leukocyte subsets and use of nicotine-rich snuff. Interestingly, the difference in lymphocytes between current smokers versus nonsmokers was larger amongst men with AAA than controls. This may be explained by a tendency amongst AAA men to smoke more cigarettes per day than controls. Further, it is possible that the AAA disease itself, or other AAA-associated confounders, may modulate the inflammatory response to cigarette smoking. Yet another possibility is that those predisposed to AAA development respond to smoking exposure with a stronger inflammatory activity. For example, carriers of IL6R genetic variants that have been associated with AAA [21] may have a heightened response to interleukin-6 triggered by tobacco smoke [12]. AAA development has been suggested to have an autoimmune component, similar to atherogenesis [13,22], and autoimmune disorders such as rheumatoid arthritis are associated with a higher risk of AAA [23]. An interesting hypothesis is that smoking may trigger both formation of and autoimmune activation against elastin fragments from lung, in accordance with

| Table 3: Multivariate-adjusted odds ratios (95% confidence intervals) for AAA in relation to categories of leukocyte subsets |
|---------------------------------------------------------------|
| Leukocyte subset     | Model 1         | Model 2         | Model 3         | Model 4         | Model 5         | Model 6         |
|----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Neutrophils          |                 |                 |                 |                 |                 |                 |
| Per Q increase       | 1.93 (1.57–2.36) | 1.74 (1.41–2.16) | 1.55 (1.23–1.97) | 1.46 (1.12–1.92) | 1.52 (1.15–2.01) | 1.45 (1.10–1.91) |
| P Value              | <0.001          | <0.001          | <0.001          | 0.006           | 0.003           | 0.009           |
| Lymphocytes          |                 |                 |                 |                 |                 |                 |
| Q4 vs. Q1-3          | 3.20 (1.96–5.24) | 2.42 (1.43–4.10) | 2.19 (1.20–4.00) | 2.15 (1.10–4.22) | 2.28 (1.14–4.54) | 2.37 (1.18–4.76) |
| P Value              | <0.001          | <0.001          | 0.011           | 0.026           | 0.020           | 0.015           |
| Monocytes            |                 |                 |                 |                 |                 |                 |
| Per Q increase       | 1.48 (1.21–1.80) | 1.34 (1.09–1.65) | 1.10 (0.87–1.40) | 1.01 (0.77–1.34) | –               | –               |
| P Value              | <0.001          | 0.006           | 0.41            | 0.92            |                 |                 |
| Eosinophils          |                 |                 |                 |                 |                 |                 |
| Q2-4 vs. Q1          | 0.59 (0.35–1.01) | 0.54 (0.31–0.93) | 0.61 (0.32–1.13) | 0.56 (0.27–1.17) | –               | –               |
| P Value              | 0.055           | 0.028           | 0.12            | 0.13            |                 |                 |
| Basophils            |                 |                 |                 |                 |                 |                 |
| >0 vs. 0             | 1.94 (1.23–3.06) | 1.61 (0.99–2.63) | 1.54 (0.90–2.65) | 1.25 (0.67–2.35) | –               | –               |
| P Value              | 0.004           | 0.053           | 0.12            | 0.49            |                 |                 |

Analysis by logistic regression. AAA indicates abdominal aortic aneurysm; Q, quartile.
Model 1: Crude model.
Model 2: Adjusted for current smoking.
Model 3: Adjusted for current smoking and pack-years (in quartiles).
Model 4: Model 3 plus adjustment for weight, height, systolic blood pressure, total cholesterol, HDL cholesterol, diabetes mellitus, use of anti-hypertensive medications, use of statins.
Model 5: Model 4 plus adjustment for prevalent asthma/COPD.
Model 6: Model 4 plus adjustment for prevalent cardiovascular disease and prevalent cerebrovascular disease.
elevated elastin (auto-)antibodies in patients with AAA [13].

Previous studies have reported an association between COPD and AAA [24]; whilst controversial, certain data suggest that this association may be independent of smoking [25]. In the present study, we could not detect a significantly increased frequency of self-reported asthma/COPD amongst men with AAA, but our study may be underpowered for this question. Notably, adjustment for asthma/COPD did not materially alter the leukocyte-AAA associations.

Our study replicates previous findings regarding factors associated with AAA, including smoking, hypertension, body weight, HDL cholesterol, use of statins and prevalent cardiovascular disease [3,4]. The higher statin use in those with screening-detected AAA may explain why serum total cholesterol was lower amongst these individuals. The prevalence of AAA was 1.2% amongst 65-year-old men screened between 2013 and 2017 in the Gothenburg area [the population we used to recruit participants for this case-control study]. For comparison, the national screening audit study covering the period 2008–2014 [2] and the first screening study in Sweden, performed in the Uppsala region from 2006 to 2009 [10] reported a prevalence of 1.5% and 1.7%, respectively. The lower prevalence reported in our current study is expected and at least partly explained by changing smoking habits in the background population [10].

To date, smoking cessation is the only efficient way to reduce AAA growth; no medication has yet shown a proven effect on AAA disease development [8,13,26]. As large aneurysms unequivocally require surgery, any pharmaceutical intervention against AAA must be initiated when aneurysms are still small. Earlier studies have mainly focused on advanced disease, whilst studies derived from population-based AAA screening are better positioned to capture processes that take place earlier in the pathogenesis, and thereby still possible to target noninvasively [13,26]. Although the present study does not support neutrophil count, lymphocyte count, or the neutrophil-to-lymphocyte ratio as useful markers to predict presence of AAA in men, counts of white blood cells and their subtypes have been shown to predict the course of cardiovascular diseases [18,19]. Amongst a multitude of research questions remaining, the predictive value of different leukocyte subsets for continued AAA growth should be addressed in future studies.

Our study has limitations, including all those that are generally associated with a cross-sectional study design. Further, the results are based on single measurements of differential leukocyte counts and we only have data on number of cells in the large leukocyte classes; data regarding subtypes (such as T or B cells), distribution amongst pro- or anti-inflammatory subtypes or functional/activation status of cells are lacking. Other limitations include self-reporting of comorbidities, that the different study participation acceptance rate amongst men with AAA and controls may introduce bias, and that the results may not be generalizable to women or men of other ages. The study also has notable strengths, including the detection of early (screening-detected) disease, nonconfounding by age and sex and that all assays were performed in one laboratory. The combination of a large population-based background cohort with an excellent coverage of invitations, high attendance rate in the study and controls recruited at the same screening site, resulted in a large, well-characterized case-control sample.

Conclusion

Several, but not all, subclasses of circulating leukocytes are associated with screening-detected AAA in 65-year-old men, which is insufficiently explained by associations with smoking and other confounders. These data show that certain leukocyte subsets in blood are associated with AAA presence before clinical presentation of the disease.

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**Conflict of interest statement**

The authors have no conflicts of interest to declare.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Crude odds ratios (95% confidence intervals) for AAA in relation to categories of leukocyte subsets.