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Leukocyte profiles across the cardiovascular disease continuum: A population-based cohort study☆

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ABSTRACT

Introduction: Inflammation plays a pivotal role across all stadia of the cardiovascular disease (CVD) continuum, i.e. non-obstructive coronary artery disease (CAD), myocardial infarction (MI), and ischemic heart failure (iHF). However, inflammation across CVD continuum has not been studied yet within one population. Therefore, we mapped leukocyte profiles across the continuum within the UK Biobank.

Methods: The UK Biobank cohort study includes > 500,000 participants aged 40 to 70 years who were recruited from 22 assessment centers across the United Kingdom from 2006 to 2010. A total of 333,218 individuals with available laboratory measurements at baseline were included in this study. These consisted of controls and individuals who had progression of CVD during follow-up (i.e. who developed CAD, MI, or iHF during follow-up). We investigated whether leukocytes and subtypes of leukocytes at baseline differed among the CVD continuum. Furthermore, we studied the possible interactions between sex and CVD on leukocytes.

Results: Of 333,218 individuals, 325,054 (97.5%) individuals were categorized as controls, and 8164 (2.5%) individuals had progression of CVD during follow-up. Of those 8164 individuals, 4552 (1.4%) developed CAD during follow-up, 2839 (0.9%) MI, and 773 (0.2%) in iHF. Compared to controls, mean leukocyte levels at baseline increased across the CVD continuum from $6.8{\cdot}10^9$ cells/L (SD $1.7{\cdot}10^9$ cells/L) to $7.7{\cdot}10^9$ cells/L (SD $1.9{\cdot}10^9$ cells/L) ($P_{trend} = 2.19{\cdot}10^{-132}$) in individuals who developed iHF. This increase mainly depended on an increase in neutrophils. Furthermore, controls with leukocyte levels in the highest quartile at baseline had a 1.44 higher chance of being diagnosed with CAD during follow-up compared with individuals with leukocyte levels in lower quartiles (OR 1.44, 95% CI 1.34–1.56 $P = 9.63{\cdot}10^{-21}$). A similar increased change was observed for neutrophils, lymphocytes, monocytes, and eosinophils. There was a significant interaction between sex and CVD continuum on lymphocytes ($P = 8.49{\cdot}10^{-5}$).

Conclusion: Overall leukocyte count increased across the CVD continuum, which mainly depended on the increase in neutrophil count. High leukocytes in individuals not having CAD at baseline were predictive for the development of CAD during follow-up. Women had a greater increase of lymphocytes across the CVD continuum compared to men. Understanding which cells are key players in which stadium, could serve as a starting point for the identification of new potential therapeutic targets in CVD.

1. Introduction

It is generally appreciated that inflammation plays a pivotal role in the pathophysiology of atherosclerosis and cardiovascular disease (CVD). This has been shown on genetic, biologic, epidemiologic and clinical trial level [1,2]. Nevertheless, these supporting data contrast with neutral clinical trials studying the effect on anti-inflammatory agents on (a composite of) major cardiovascular events, emphasizing the complexity of the human immune system and the need for more studies unravelling this complex mechanism [3]. Recently, the CANTOS trial showed improved CVD outcomes using the anti-interleukin-1β antibody canakinumab, although CVD mortality did not improve and there was an increase in the rate of infections. Therefore, optimization of immunotherapy for CVD is required (i.e. identification of novel drug targets that block inflammatory pathways, but do not exhibit immune-suppressive side effects). In order to achieve this optimization it is of value to first obtain an overview of the immune response in CVD patients. This has been done in the European Prospective Investigation

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into Cancer in Norfolk (EPIC-Norfolk) study, which studied differential white blood cell count in approximately sixteen thousand apparently healthy individuals [4,5]. They observed associations between increased leukocytes and the risk for CVD. However, leukocyte profiles in different conditions of CVD (i.e. coronary artery disease (CAD), myocardial infarction (MI), and ischemic heart failure (iHF)), also known as the cardiovascular disease continuum (CVD continuum) [6], within one population have not been studied yet. In combination with an increased sample size, information on the different components of granulocytes (i.e. neutrophils, eosinophils, and basophils), and further exploration of potential sex differences, this might be of additive value to the existing data from the EPIC-Norfolk study. Understanding which immune cells are present in particular stadia of the continuum may aid in increasing our knowledge of the underlying pathophysiologic process and expanding our efforts of optimising therapies. Therefore, we aimed to study leukocyte profiles in different stadia of the CVD continuum and to provide a broader overview of associations between leukocytes and CVD in individuals participating in the UK Biobank.

2. Methods

2.1. UK Biobank participants

The UK Biobank study design and population have been described in detail elsewhere [7]. In brief, UK Biobank is a large community-based prospective study in the United Kingdom that recruited > 500,000 participants aged 40 to 69 years old with the aim of improving prevention, diagnosis, and treatment of a plethora of illnesses including cancer, diabetes mellitus, stroke, and heart diseases. All participants gave informed consent for the study. UK Biobank has approval from the relevant institutional review boards, namely, the North West Multicentre Research Ethics Committee for the UK, from the National Information Governance Board for Health & Social Care for England and Wales, and from the Community Health Index Advisory Group for Scotland [8]. The present study was conducted under application number 12006 of the UK Biobank resource. The data from the UK Biobank resource are available for other researchers following an approved research proposal [9]. We used self-reported diagnoses and medication, and Hospital Episode Statistics data, as previously described [10]. We excluded individuals with a non-Caucasian ethnicity (n = 29,750), missing laboratory values (n = 21,960), and disease and/or therapy at baseline present which could affect cell counts (n = 117, 631). This included: C-reactive protein ≥10 mg/L (n = 37,908), HIV (n = 15), malignant cancer (n = 64,351), immunosuppressants/chemotherapy (n = 3373), rheumatoid arthritis (n = 3650), rheumatic heart disease (2347), and gout (n = 5987). We created a set of 333,218 individuals. Fig. 1 shows a flowchart of the study sample selection.

2.2. Definition of new-onset cardiovascular diseases

For our analyses we included individuals who had progression of CVD during follow-up. We used the following categories: controls, individuals who developed CAD during follow-up, individuals who developed MI during follow-up, and individuals who developed iHF during follow-up. Explanation of CVD continuum categories can be found in Supplementary file 1. Individuals were only placed in one category. When individuals initially could belong to more categories, they were placed in the 'worst' category.

Follow-up for disease outcomes was censored on 31-03-2015 for participants from England, 31-08-2014 for participants from Scotland, and on 28-02-2015 for participants from Wales.

2.3. Vital signs and blood count

At the baseline visit, vital signs and biological samples (i.e. prior to the progression of CVD during follow-up) were collected, together with data of self-completed questionnaires, interviews, and physical measurements. Blood pressure was measured twice, automated or manually, and average values were used. Automated measurements were corrected as proposed by Stang et al [11].

2.4. Statistical analysis

Continuous variables were summarized as mean ± standard deviation if normally distributed or median and interquartile range if skewed. Discrete variables were presented as frequencies and percentages. We performed linear regression analysis to study the association between the different stages of the CVD continuum and cells. Age, sex, and CVD continuum stage were included in multivariate regression analysis. Afterwards, we performed post-hoc analyses to study trends across the CVD continuum and to study sex interactions with the CVD continuum on leukocytes. To validate our findings, we repeated the linear regression analyses with a smaller set of controls, based on propensity score matching (age and sex) to the MI individuals, since the MI individuals had the average age and male percentage of the CAD, MI, and iHF group combined. Logistic regression analysis was performed to assess the chance of being diagnosed with CAD, MI or iHF during follow-up compared to a stage less worse at baseline. We adjusted for age and sex. We used Mann-Whitney to test for differences in leukocyte profiles between iHF and non-ischemic HF. To maximize the likelihood of reporting true findings, we set the α at 0.005 instead of 0.05 and used Bonferroni correction to adjust for multiple testing [12]. We considered 2-sided P values $< 1.39{\cdot}10^{-4}$ (P value of < 0.005 divided by the number of independent tests, calculated using the Nyholt method, ie, 0.005/36) statistically significant for all analyses [13,14]. P values $< 1.39{\cdot}10^{-3}$ (ie, 0.05/36) were considered of suggestive significance. All analyses were performed using Stata version 15 (StataCorp).

3. Results

3.1. Population characteristics

From the 502,559 individuals, we included a total of 333,218 individuals for the present analyses (Fig. 1). Overall, 153,043 (46%) were male. Mean age was 57 years (SD 8 years) and median follow-up of 6 years (interquartile range [IQR] 5–7). Baseline characteristics, including cardiovascular risk factors and medical history of coronary heart diseases are provided in Table 1.

3.2. Leukocytes increase across the CVD continuum

Of 333,218 individuals, 325,054 (97.5%) individuals were categorized as controls and 8164 (2.5%) individuals had progression of CVD during follow-up. Of those 8164 individuals, 4552 (1.4%) developed CAD, 2839 (0.9%) MI, and 773 (0.2%) in iHF. The absolute and relative number of all leukocytes is shown in Table 2. Compared to controls, mean leukocyte levels at baseline increased across the CVD continuum from $6.8{\cdot}10^9$ cells/L (SD $1.7{\cdot}10^9$ cells/L) to $7.7{\cdot}10^9$ cells/L (SD $1.9{\cdot}10^9$ cells/L) ($P_{\text{trend}} = 2.19{\cdot}10^{-132}$) in individuals who developed iHF during follow-up. Baseline neutrophil count increased across the continuum with a range from $4.2{\cdot}10^9$ cells/L (SD $1.3 \cdot 10^9$ cells/L) in controls to $4.9{\cdot}10^9$ cells/L (SD $1.5{\cdot}10^9$ cells/L) in individuals who developed iHF during follow-up ($P_{trend} = 4.65{\cdot}10^{-112}$). Fig. 2 shows the association between CVD continuum categories and leukocyte and neutrophil counts. After adjustment for age and sex, being in a 'worse' CVD continuum category during follow-up remained significantly associated with an increase in total leukocytes and neutrophils compared to controls. A similar phenomenon was observed for absolute cell counts of lymphocytes, monocytes, eosinophils, and basophils (Supplementary Table 2), and also in the analyses using the control group

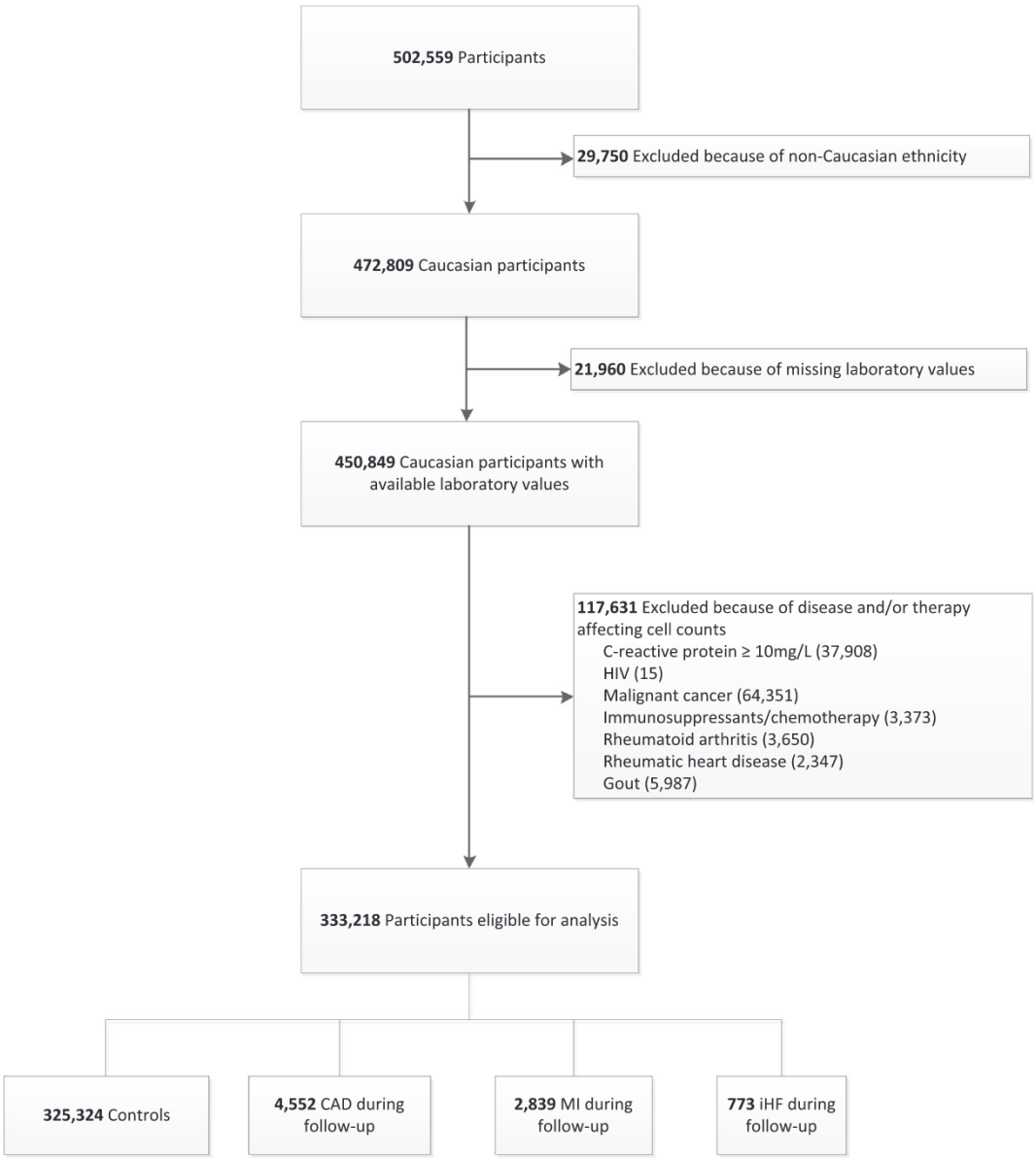

Fig. 1. Flowchart for the selection of the analysed study sample from the UK Biobank study.

(n = 2837) based on propensity score matching (Supplementary Table 3).

3.3. Prognostic value of leukocytes for progression CVD

Furthermore, we investigated the predictive value of high leukocytes levels at baseline in the progression of CVD during follow-up (Fig. 3, Table 3). We defined this progression as a change in category between baseline and follow-up (i.e. no CAD to CAD, CAD to MI, MI to iHF). Individuals without CAD with leukocyte levels in the highest quartile at baseline had a 1.44 higher chance of being diagnosed with CAD during follow-up compared with individuals with leukocyte levels in lower quartiles (OR 1.44, 95% CI 1.34–1.56 $P = 9.63{\cdot}10^{-21}$). A similar increased change was observed for neutrophils, lymphocytes, monocytes, and eosinophils. This increased chance was not observed for individuals with progression of their CVD in a later stadium (CAD to MI, or MI to iHF) (Table 3).

To see whether the increase in leukocyte levels was really related to the inflammatory and ischemic component of the CVD continuum, we studied the differences in levels between iHF and non-ischemic HF individuals. Level of leukocytes, lymphocytes, and monocytes were significantly higher in individuals with iHF compared with individuals with non-ischemic HF (Supplementary Table 4).

To explore whether the increase in leukocyte levels across the CVD continuum would reflect a difference in functionality of the immune system, we investigated whether there was a difference in pneumonia incidence during follow-up between controls and individuals with iHF. In the iHF group, the incidence of pneumonia during follow-up was significantly higher compared to controls (13.8% vs. 1.0%, $P < 5.00{\cdot}10^{-324}$) (Fig. 4). This difference remained in the analysis using the control group (n = 2837) based on propensity score matching.

3.4. Sex differences in leukocyte profiles

Overall, women had higher leukocyte counts compared to men ($P = 8.04{\cdot}10^{-}5$) (Supplementary Table 5). In addition, men had relatively higher levels of neutrophils, monocytes, and eosinophils, whereas women had relatively higher levels of lymphocytes and basophils. Furthermore, we investigated whether there were any interaction

Table 1
Baseline characteristics.

Characteristic	Controls	CAD	MI	iHF
Total, no.	325,054	4552	2839	773
Male	147,427 (45.4%)	2949 (64.8%)	2060 (72.6%)	607 (78.5%)
Age, mean (SD), y	56.4 (8.1)	61.0 (6.5)	60.1 (6.8)	62.4 (6.1)
Blood pressure, mean (SD), mmHg				
Systolic	132.6 (17.8)	140.6 (17.8)	142.0 (18.2)	140.2 (19.2)
Diastolic	81.9 (8.5)	83.7 (8.6)	84.9 (8.9)	81.8 (9.5)
Heart rate, beats per minute	68.9 (11.0)	69.0 (11.7)	69.6 (12.2)	67.9 (13.4)
Smoking				
Never or < 100 cigarettes	183,337 (57.8%)	2084 (46.9%)	1173 (42.2%)	252 (33.4%)
Stopped > 12 months	100,243 (31.6%)	1823 (41.0%)	1008 (36.2%)	318 (42.2%)
Stopped ≤12 months	1427 (0.4%)	22 (0.5%)	22 (0.8%)	13 (1.7%)
Active ocassionally	8579 (2.7%)	95 (2.1%)	95 (3.4%)	32 (4.2%)
Active daily	23,642 (7.5%)	424 (9.5%)	484 (17.4%)	139 (18.4%)
Body mass index, kg/m^2	27.1 (4.5)	28.6 (4.7)	28.3 (4.4)	29.6 (5.2)
Hypertension	85,950 (26.4%)	2231 (49.0%)	1262 (44.5%)	551 (71.3%)
Hyperlipidemia	54,354 (16.7%)	1656 (36.4%)	879 (31.0%)	510 (66.0%)
Type 2 Diabetes	8261 (2.5%)	436 (9.6%)	230 (8.1%)	174 (22.5%)

Data is expressed as number (%) and as mean (standard deviation (SD)) for a normal distribution. CAD = coronary artery disease; MI = myocardial infarction; iHF = ischemic heart failure.

between sex and leukocytes. A significant overall interaction between CVD continuum and sex was observed for lymphocytes, where these increased more in women than in men ($P = 8.49{\cdot}10^{-5}$).

4. Discussion

In this large community-based population of > 330,000 individuals, we mapped leukocyte profiles across the different stadia of the CVD continuum (CAD, MI, and iHF). First, overall leukocyte count increased across the continuum, which mainly depended on the increase in neutrophil count. Second, high levels of leukocytes at baseline were predictive for the progression of CVD during follow-up. Third, women showed overall higher leukocyte levels and there was an interaction between CVD continuum and sex on lymphocytes, suggesting a sex specific component in leukocyte profiles across the CVD continuum.

4.1. Leukocyte profiles across the CVD continuum

Across the CVD continuum, we observed an increase in leukocyte count, mainly depending on the increase in neutrophils. This might indicate that neutrophils alone may account for more vascular damage than any other single cell type, as they are often the first cells which are recruited into the damage site [15,16]. We observed the largest increase in neutrophil count between controls and individuals who developed CAD during follow-up, but this increase continues in individuals who developed MI and iHF during follow-up, supporting that the immune system plays a role across all stages of the CVD continuum [1]. The phenomenon of the increasing neutrophils across the different stages of the CVD continuum is also in line with previous research that showed that an increased ratio between neutrophils and lymphocytes is an independent predictor of cardiac morality in stable CAD patients [17]. The possibility to show this increase in neutrophils across the different CVD continuum stages is of additive value, since it enables to obtain the bigger picture. Furthermore, our results support earlier research that postulated that neutrophil extracellular traps (NETs) are not only present in plaques and thrombi, but also may play a causative role in triggering atherosclerotic plaque formation and arterial thrombosis [18,19]. Of interest is the predictive value of increased leukocyte levels at baseline on the progression of CAD during a six years follow-up. This association did not remain in individuals with either CAD or MI at baseline, and getting diagnosed with MI or iHF during follow-up. Although this finding is solely observational and suggestively significant, our study is of additional value to earlier results of the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) study, that also showed the association between increased leukocytes and a higher risk for coronary heart disease and CVD, and might implicate that early intervention in the inflammation pathway is of importance to gain as much effect as possible [3,4].

Furthermore, we observed higher leukocyte levels in iHF individuals compared with non-ischemic HF individuals. Levels in both groups were higher compared to controls. Additionally, the incidence of pneumonia was fourteen-fold higher in the iHF group compared to

Table 2
Leukocyte profiles across the cardiovascular disease continuum.

	Controls	CAD	MI	iHF	*P* value for trend
Number	325,054	4552	2839	773	
Leukocyte count, 10^9 cells/L	6.8 (1.7)	7.1 (1.8)	7.3 (1.9)	7.7 (1.9)	$2.19{\cdot}10^{-132}$
Neutrophil count, 10^9 cells/L	4.2 (1.3)	4.4 (1.4)	4.6 (1.5)	4.9 (1.5)	$4.65{\cdot}10^{-112}$
Percentage of neutrophils, %	60.8 (8.1)	61.0 (8.1)	61.6 (8.1)	62.8 (8.5)	$6.91{\cdot}10^{-15}$
Lymphocyte count, 10^9 cells/L	0.9 (0.4)	0.9 (0.5)	0.9 (0.5)	0.9 (0.5)	$1.24{\cdot}10^{-28}$
Percentage of lymphocytes, %	29.0 (7.1)	28.4 (7.1)	27.8 (7.0)	26.3 (7.2)	$6.45{\cdot}10^{-22}$
Monocyte count, 10^9 cells/L	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.6 (0.2)	$1.59{\cdot}10^{-43}$
Percentage of monocytes, %	7.1 (2.6)	7.3 (2.5)	7.3 (2.5)	7.6 (3.3)	0.164
Eosinophil count, 10^9 cells/L	0.13 (0.10–0.20)	0.16 (0.10–0.23)	0.18 (0.10–0.25)	0.19 (0.10–0.25)	$3.64{\cdot}10^{-33}$
Percentage of eosinophils, %	2.11 (1.38–3.27)	2.27 (1.50–3.40)	2.31 (1.52–3.47)	2.24 (1.15–3.29)	$1.00{\cdot}10^{-4}$
Basophil count, 10^9 cells/L	0.02 (0.00–0.04)	0.02 (0.00–0.04)	0.02 (0.00–0.05)	0.03 (0.00–0.06)	$1.56{\cdot}10^{-36}$
Percentage of basophils, %	0.43 (0.30–0.67)	0.43 (0.30–0.66)	0.41 (0.30–0.65)	0.47 (0.30–0.70)	0.060

Data is expressed as mean with standard deviation (SD) or median with interquartile range (IQR). CAD = coronary artery disease; MI = myocardial infarction; iHF = ischemic heart failure. *P* value for trend was derived from the post-hoc linear regression analysis.

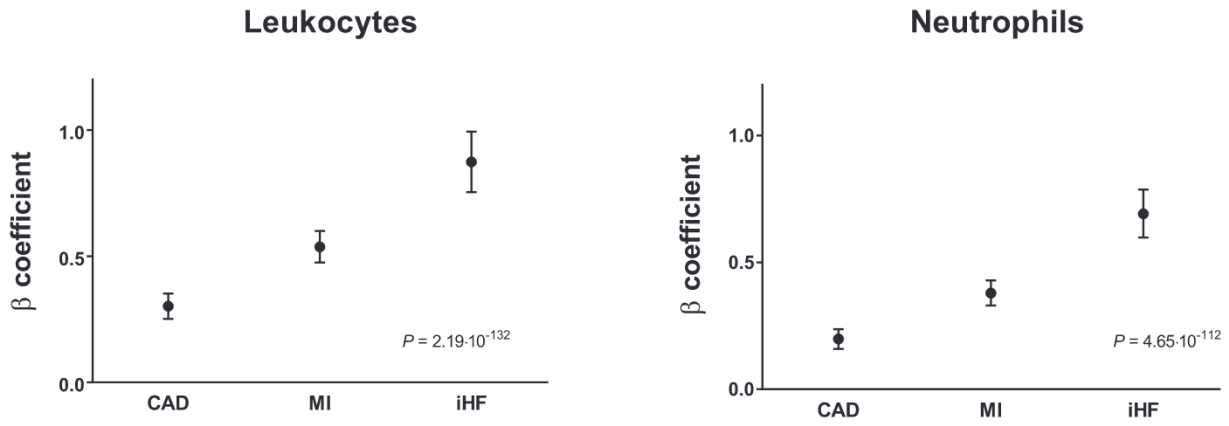

Fig. 2. Associations between CVD continuum and leukocytes, neutrophils, and monocytes, corrected for age and sex. CAD = coronary artery disease; MI = myocardial infarction; iHF = ischemic heart failure.

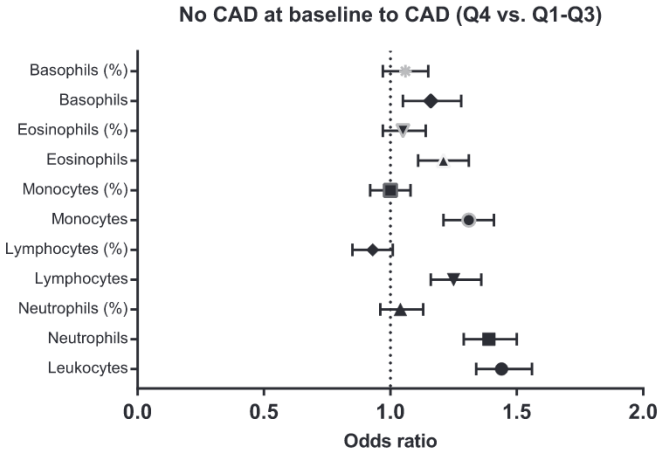

Fig. 3. Chance of getting diagnosed with CAD during follow-up, when having no CAD at baseline. Highest quartile of leukocytes (Q4) vs. lower quartiles (Q1 – Q3). CAD = coronary artery disease.

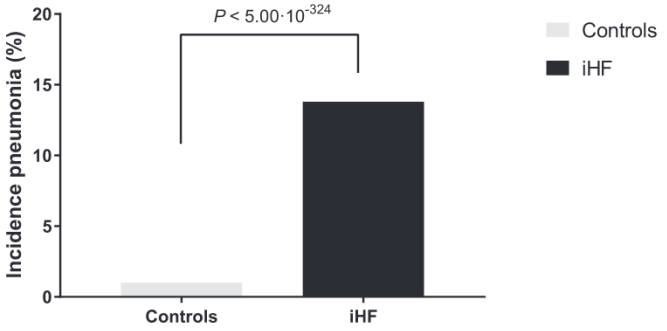

Fig. 4. Incidence of pneumonia during follow-up in controls and individuals with iHF. iHF = ischemic heart failure.

controls, which may also point to a functional change of the leukocytes. Although data on the functionality of leukocytes was not available and there might be other explanations for this difference (i.e. presence of pulmonary oedema), this finding supports the need for prophylactic vaccination in iHF individuals.

4.2. Sex differences

It is generally appreciated that sex differences exist in the innate and adaptive immune response in adults [20]. In this study, sex differences were also observed. Women showed higher levels of total leukocytes, and specifically lymphocytes and basophils. The opposite was observed for neutrophils, monocytes, and eosinophils, which were higher in men compared with women. Furthermore, of interest is the interactions between sex and CVD continuum on lymphocytes. T-lymphocytes are known to stimulate macrophages expressing collagen-degrading enzymes and thereby increasing the risk of plaque rupture [21]. Estrogen has been shown to be a cardioprotective agent in pre-menopausal females, partially by reducing atherosclerosis via the estrogen receptor α [22]. Although highly speculative, it might be hypothesized that the increase in lymphocytes in women compared to men might reflect that women are less prone to inflammation (due to the protective function of estrogen), before they develop MI [23]. Estimating lymphocytes in pre-

Table 3
The chance of being diagnosed with progression across the CVD continuum during follow-up: individuals with leukocyte levels in the highest quartile (Q4) compared with individuals with leukocyte levels in the lower quartiles ((Q1 – Q3).

	No CAD at baseline to CAD	*P* value	CAD at baseline to MI	*P* value	MI at baseline to iHF	*P* value
Leukocyte count (Q4 vs. Q1–3)	1.44 (1.34–1.56)	$9.63{\cdot}10^{-21}$	1.43 (1.14–1.80)	0.002	1.26 (1.02–1.55)	0.032
Neutrophil count (Q4 vs. Q1–3)	1.39 (1.29–1.50)	$1.58{\cdot}10^{-16}$	1.49 (1.19–1.87)	0.001	1.43 (1.16–1.76)	0.001
Percentage of neutrophils (Q4 vs. Q1–3)	1.04 (0.96–1.13)	0.330	1.21 (0.95–1.54)	0.115	1.33 (1.07–1.66)	0.010
Lymphocyte count (Q4 vs. Q1–3)	1.25 (1.16–1.36)	$3.20{\cdot}10^{-8}$	1.07 (0.83–1.39)	0.591	0.94 (0.75–1.19)	0.620
Percentage of lymphocytes (Q4 vs. Q1–3)	0.93 (0.85–1.01)	0.089	0.79 (0.58–1.08)	0.133	0.66 (0.48–0.89)	0.008
Monocyte count (Q4 vs. Q1–3)	1.31 (1.21–1.41)	$2.39{\cdot}10^{-11}$	1.24 (0.99–1.55)	0.066	1.41 (1.14–1.74)	0.001
Percentage of monocytes (Q4 vs. Q1–3)	1.00 (0.92–1.08)	0.984	1.19 (0.94–1.50)	0.152	1.15 (0.93–1.43)	0.187
Eosinophil count (Q4 vs. Q1–3)	1.21 (1.11–1.31)	$5.12{\cdot}10^{-6}$	1.09 (0.86–1.39)	0.454	1.10 (0.89–1.37)	0.373
Percentage of eosinophils (Q4 vs. Q1–3)	1.05 (0.97–1.14)	0.205	0.98 (0.77–1.26)	0.855	0.94 (0.75–1.18)	0.606
Basophil count (Q4 vs. Q1–3)	1.16 (1.05–1.28)	0.003	1.40 (1.06–1.86)	0.018	1.52 (1.18–1.95)	0.001
Percentage of basophils (Q4 vs. Q1–3)	1.06 (0.97–1.15)	0.177	1.12 (0.86–0.45)	0.406	1.36 (1.08–1.72)	0.010

Data is expressed as odds ratio (OR) and 95% Confidence Interval (CI). CAD = coronary artery disease; MI = Myocardial infarction; iHF = ischemic heart failure. Logistic regression was performed, adjusted for age and sex.

and post-menopausal women in the UK Biobank did, however, not provide additional support. On the other hand, in a study of 6050 patients that underwent coronary computed tomography angiography, women had a higher relative risk for high-risk plaque features compared to men, which might correspond with higher lymphocyte levels [24]. Nevertheless, the difference in lymphocyte trends between women and men across the CVD continuum invites to further investigate potential mechanisms underlying. Furthermore, the difference in leukocytes between men and women may also cohere with the a difference in gene expression between sexes. Previous research showed a greater upregulation of the gene *FAM5C* in women [23]. *FAM5C* is associated with increased monocyte adhesion, suggesting that women may need less monocytes to experience the similar amount of monocyte adhesion compared to men [23]. The increased association in both men and women between the CVD continuum and monocytes is intriguing, since this observation is contrary to previous research in the EPIC-Norfolk study [5]. They found an inverse relation between monocytes and the risk of developing HF, whereas we observed an increased association between CVD continuum and monocytes. This could be due to another definition of HF, where they based HF on drug treatment only and we tried to focus on ischemic HF solely. Nevertheless this finding warrants further exploration of the underlying mechanism.

4.3. Strengths and limitations

In this study, we were able to provide an elegant overview of the different leukocyte profiles across the CVD continuum within one population. The major strengths of this study were the large sample size, variety of information, and prospective design of the UK Biobank study. Furthermore, in addition to the EPIC-Norfolk study, we did have information on the components of granulocytes (neutrophils, eosinophils, basophils) to further study their specific effect [4].

However, this study possesses some limitations. We only had information on the quantity of the cells. It would be of value to also possess information on the functionality and activity of cells, and cytokine levels, since this could provide new insights into the cellular cross-talk between immune cells and their related pathways [25]. Nevertheless, this study could serve as a starting point, providing new insights where to focus concerning new possible therapeutic targets.

4.4. Future perspectives

Single-cell RNA sequencing data of immune cells in different stages of the CVD continuum might be a potential way to gain more insights into the inflammatory pathophysiological mechanisms underlying CVD, as this provides additional information on the cell-cell interactions in the heart. Until now, the cellular interactions and their dynamics during the different stages of the CVD continuum remain poorly characterized [26]. This method has already been performed in animals and in healthy humans, but has not yet been applied to cardiovascular patients [26,27]. Furthermore, we provided evidence for sex differences in leukocyte profiles across the CVD continuum. Further research on these differences is worthwhile for the development of more patient-tailored therapies.

5. Conclusions

Our study provided an overview of the different leukocyte profiles across different stadia of the CVD continuum. Overall leukocyte count increased across the continuum, which mainly depended on the increase in neutrophil count. Furthermore, high levels of leukocytes at baseline were predictive for the progression of CVD during follow-up. Last, women showed overall higher leukocyte levels and there was an interaction between CVD continuum and sex on lymphocytes, suggesting a sex specific component in leukocyte profiles across the CVD continuum. Understanding which cells are key players in various stadia, could serve as a starting point for the identification of new potential therapeutic targets in CVD.

Explanation of the role of funder/sponsor

The funding agencies had no role in the study design, analysis, or interpretation of data; the writing of the manuscript; or in the decision to submit the article for publication.

Data statement

The data, analytical methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure (contact the corresponding author).

Declaration of Competing Interest

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yjmcc.2019.11.156.

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