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Commonly available hematological biomarkers are associated with the extent of coronary calcifications

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ABSTRACT

Background and aims: We aimed to improve the understanding of potential associations between commonly available hematological biomarkers and the coronary artery calcification (CAC) score, which may help unravel the pathophysiology of coronary calcifications and subclinical coronary artery disease.
Methods: A cross-sectional study was performed within the Utrecht Patient Oriented Database (UPOD). Patients with suspected or known coronary artery disease who underwent CT CAC scoring as well as standard hematology analysis that was part of routine clinical care (within 3 months of CT acquisition) were included. Complete hematology datasets were extracted from hematology analyzers. Linear regression adjusted for potential confounders was used to assess if hematological biomarkers were related to the CAC score.
Results: In total, 1504 patients were included, of whom 43% (n = 647) had a CAC score of 0. Mean age (±SD) was 53 ± 13 years, and 34% of patients were women. Red blood cell distribution width (RDW, β = 0.20 [0.05–0.36], p=0.007), the fraction of immature reticulocytes (β = 0.97 [0.10–6.43], p=0.004), coefficient of variation of neutrophil lobularity (β = 0.13 [0.01–0.25], p=0.040) and mean lymphocyte cell size (β = 0.21 [0.08–0.34], p=0.001) were positively associated with the CAC score after adjustment for age, sex, body mass index (BMI), diabetes, glomerular filtration rate (GFR) and high-density lipoprotein (HDL).
Conclusions: This study confirms the known association of RDW with the CAC score, and presents the fraction of immature reticulocytes, coefficient of variation of neutrophil lobularity, and mean lymphocyte cell size as new markers associated with a higher CAC score.

1. Introduction

The most common cause of death globally is coronary artery disease [1]. The coronary artery calcification (CAC) score is an established marker of coronary artery disease and it reflects the burden of atherosclerosis. The CAC score can therefore serve as a marker for the presence and extent of atherosclerosis. It can be determined in a standardized non-contrast-enhanced computed tomography (CT) acquisition and expresses the amount of calcification in the coronary arteries. High CAC scores are associated with an increased risk of cardiovascular events and mortality [2]. The exact mechanism of the formation of these atherosclerotic calcifications is not well understood. Proteins involved in bone formation, such as osteopontin, are thought to play a role in the process of coronary artery calcification [3,4]. It is known that atherosclerosis is

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a chronic inflammatory disease [5], and studies investigating the role of blood cells have therefore primarily focused on associations between leukocytes and coronary artery disease [6–12]. A high total leukocyte count is associated with cardiovascular disease [8]. Furthermore, the recent CANTOS trial showed that anti-inflammatory therapy can reduce the rate of cardiovascular events compared to placebo [13]. Also, aberrant hematopoiesis is associated with atherosclerosis [14]. However, data regarding the morphology of hematologic cells as well as studies investigating the role of red blood cells and platelets are scarce, despite the well-known role of the latter in atherosclerotic and atherothrombosis. The red blood cell distribution width (RDW) is the best known morphologic biomarker that is associated with coronary artery disease [15,16]. Modern hematology analyzers automatically measure blood cell morphology biomarkers when performing a complete blood count [17]. However, these biomarkers are usually not extracted nor reported to the clinic and therefore not used for diagnostic or therapeutic purposes. In the Utrecht Patient Oriented Database, these background data on blood cell morphology have been routinely extracted into the database since 2005 for every blood sample routinely measured in our laboratory. More information about the association between hematologic biomarkers and the CAC score might improve our understanding of the role of blood cells in CAC. Therefore, the goal of this study was to investigate the association between hematologic biomarkers and the presence and extent of atherosclerosis as reflected by the CAC score in a large group of patients.

2. Patients and methods

2.1. Study population and design

A cross-sectional study was conducted at a tertiary referral center in the Netherlands (University Medical Center Utrecht). The local institutional review board waived the need for informed consent (protocol number 14-586/C). Patients with suspected or known coronary artery disease who underwent coronary calcium scoring between June 2011 and June 2016 were eligible for inclusion. Patients with recent hematological data, defined as maximal three months (91 days) before or after CT scanning, were included. In case multiple CAC scores or hematological datasets were available, the measurements with the shortest time between the CT acquisition and hematological data was selected.

2.2. Coronary artery calcium scoring

All acquisitions were performed on a 256-slice CT system (iCT, Philips Healthcare, Best, The Netherlands) using the routine clinical care protocol. Beta blockers were administered to the patient if the heart rate was higher than 70 beats per minute. A prospective ECG-triggered acquisition was performed during the diastolic phase. The systolic phase was used in case of an irregular heart rate. Tube voltage was set to 120 kVp and tube current was 40 mAs for patients up to 80 kg, and 50 mAs for patients $\geq$80 kg. Images were reconstructed at a slice thickness of 3.0 mm. The CAC score was quantified by a trained technician using commercially available software (Heartbeat CS, Philips Healthcare, The Netherlands). The total CAC score was automatically extracted from the CT reports using text data mining and manually checked by one observer (AH). The CAC score was categorized as normal (0), low (1-10), low-intermediate (11-100), intermediate (101-400) or high (>400) [18–20].

2.3. Clinical characteristics

For this study, data from the Utrecht Patient Oriented Database were used. The database comprises data collected in the routine clinical care process on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders and laboratory tests of all patients treated at the University Medical Centre Utrecht since 2004. The University Medical Centre Utrecht is a 1042-bed academic teaching hospital in the centre of the Netherlands, with about 28,000 clinical and 15,000 day-care hospitalizations and 334,000 outpatient visits annually. Data acquisition and management is in accordance with the guidance of the local Institutional Review Board and the data protection officer. All data were registered during routine clinical care and no additional data were collected nor were examinations performed. Patients admitted to the hospital are informed that their data can be used for scientific research, and have the possibility to deny or withdraw permission to use their data for research. The structure and content of the Utrecht Patient Oriented Database have been described in more detail elsewhere [21].

In this study, the Cell-Dyn Sapphire blood cell analyser (Abbott Diagnostics, Lake Forest, California, USA) was used. This automatic blood cell analyser reports red blood cell, leukocyte and platelet numbers as well as morphological data. Acquisition of the morphological parameters of the cells is described in detail elsewhere [22]. The cell size was measured as well as RNA content (red blood cells), complexity and granularity (leukocytes and platelets), nuclear lobularity (leukocytes), depolarization (leukocytes) and viability (leukocytes). A list of all biomarkers is provided in Supplementary Data 1. Furthermore, the following characteristics were retrieved from the electronic health records: age (years, at the day of the CT examination), sex, body mass index (BMI, kg/m^2), diabetes, total cholesterol (mmol/L), high-density lipoprotein (HDL, mmol/L), low-density lipoprotein (LDL-C, mmol/L), triglycerides (mmol/L), statin use, use of antihypertensive agents, c-reactive protein (CRP, mg/L), glomerular filtration rate (GFR, ml/min/1.72 m^2) and smoking status (current, past, never). BMI values were obtained no more than one year before or after the CT was performed. Patients were classified as having diabetes (type I or II) either based on the use of antidiabetic medication or a recorded diagnosis of diabetes in the patient file or both. Cholesterol, HDL, LDL-C, triglycerides, CRP and GFR were acquired within three months (91 days) before or after the CT was performed. In case of multiple measurements, the measurements with the shortest time to the CT acquisition was selected.

2.4. Statistical analysis

Statistical analysis was performed using RStudio version 1.0.136 (RStudio Team, Boston, USA) and R version 3.2.4. (R Foundation for Statistical Computing, Austria). First, mutual correlation between hematological biomarkers was studied using a heatmap (R package 'gplots'). From each visually identified cluster of correlated biomarkers, the biomarker with the strongest correlation to the CAC score was selected for further analysis while the remaining biomarker(s) in the cluster were excluded. Linear regression was performed with the natural log-transformation of the (CAC score + 1) as dependent variable. Unadjusted and adjusted linear regression was performed with each biomarker in a separate model. Linear regression models were adjusted for age as well as for a set of potential confounders (age, sex, BMI, diabetes, GFR and HDL-C). The potential confounders were selected based on their known relationship with both the CAC and the hematologic system. Due to a large and not random number of missing data for smoking, this was not included as a potential confounder. However, subgroup