RESEARCH ARTICLE

Routinely measured hematological parameters and prediction of recurrent vascular events in patients with clinically manifest vascular disease

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Abbreviations: SRS, Second Manifestations of ARTerial Disease Study Risk Score; cNRI, Continuous Net Reclassification Improvement

Abstract

Background and aims

The predictive value of traditional risk factors for vascular events in patients with manifest vascular disease is limited, underscoring the need for novel biomarkers to improve risk stratification. Since hematological parameters are routinely assessed in clinical practice, they are readily available candidates.

Methods

We used data from 3,922 vascular patients, who participated in the Second Manifestations of ARTerial Disease (SMART) study. We first investigated associations between recurrent vascular events and 22 hematological parameters, obtained from the Utrecht Patient Oriented Database (UPOD), and then assessed whether parameters associated with outcome improved risk prediction.

Results

After adjustment for all SMART risk score (SRS) variables, lymphocyte %, neutrophil count, neutrophil % and red cell distribution width (RDW) were significantly associated with vascular events. When individually added to the SRS, lymphocyte % improved prediction of recurrent vascular events with a continuous net reclassification improvement (cNRI) of 17.4% [95% CI: 2.1, 32.1%] and an increase in c-statistic of 0.011 [0.000, 0.022]. The combination

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of lymphocyte % and neutrophil count resulted in a cNRI of 22.2% [3.2, 33.4%] and improved c-statistic by 0.011 [95% CI: 0.000, 0.022]. Lymphocyte % and RDW yielded a cNRI of 18.7% [3.3, 31.9%] and improved c-statistic by 0.016 [0.004, 0.028]. However, the addition of hematological parameters only modestly increased risk estimates for patients with an event during follow-up.

Conclusions

Several hematological parameters were independently associated with recurrent vascular events. Lymphocyte % alone and in combination with other parameters enhanced discrimination and reclassification. However, the incremental value for patients with a recurrent event was limited.

Introduction

The most common underlying cause of cardiovascular disease is atherosclerosis, leading to over 13 million deaths per year worldwide [1]. The implementation of preventive therapies critically depends on the reliable identification of individuals at risk. In clinical practice, vascular risk assessment is primarily based on risk factors, such as smoking, hypertension, diabetes, obesity and hyperlipidemia [2]. While a large body of evidence has underpinned the significance of such traditional risk factors in primary prevention [3–5], their predictive value for vascular risk in patients with established vascular disease is less clear [6–8]. Thus, novel risk factors are needed to improve risk stratification in secondary prevention and to establish the pathophysiological processes underlying recurrent vascular risk.

The SMART risk score (SRS) has been specifically developed to predict recurrent vascular events in patients with established atherosclerotic vascular disease [9]. This score not only includes traditional risk factors, but also vascular disease history, renal function and high-sensitive C-reactive protein (hs-CRP), an inflammatory marker associated with vascular risk [10]. Besides hs-CRP, several other biomarkers have been linked to prognosis of vascular disease, including N-terminal pro-type brain natriuretic peptide, troponins, ST2 and growth-differentiation factor-15 [6,11]. A recent study identified different routinely-measured hematological parameters that predict outcomes in patients with coronary artery disease [12]. Because these parameters are measured by most hematology analyzers, they are readily available for use in clinical practice without the need to rely on expensive equipment. Despite their potential clinical utility, no study has yet assessed whether hematological parameters improve prediction of recurrent events beyond established secondary risk factors used in the SRS. Combining data from the Second Manifestations of ARTerial Disease (SMART) study and the Utrecht Patient Oriented Database (UPOD), we investigated the incremental value of routinely measured hematological parameters for the prediction of recurrent vascular events. We first investigated associations between 22 hematological parameters and recurrent vascular events. Then, we assessed whether parameters independently associated with recurrent events improved risk prediction compared to the SRS.

Methods

Study population

We conducted this study in patients with a clinical manifestation of atherosclerotic vascular disease (cerebrovascular disease, coronary artery disease, peripheral artery disease or
abdominal aortic aneurysm) who participated in the SMART study. Details on disease definitions and recruitment procedures have been published previously [9,13]. Briefly, the SMART study, an ongoing, single-center, prospective cohort study, enrolled patients aged 18–80 who were referred to the University Medical Center Utrecht for clinical manifestations of atherosclerotic vascular disease or the treatment of vascular risk factors. Because complete hematological parameters were not available before 2005, we restricted our analysis to a subset of patients enrolled from January 2005 onwards. For this study, follow-up data were available until March, 2014. At baseline, patients were requested to fill in a questionnaire on medical history, symptoms of vascular disease and vascular risk factors. During follow-up, questionnaires were sent to patients or their general practitioner twice a year to obtain information on their health status. Moreover, hospital discharge letters were collected to verify vascular events. All events were adjudicated by three members of the Endpoint Committee. The outcome of interest was a composite endpoint of vascular death, ischemic or hemorrhagic stroke or myocardial infarction, as previously described in more detail [9]. All patients provided written informed consent. The SMART study was approved by the Ethics Committee of the University Medical Center Utrecht.

**Hematological parameters**

We enriched the SMART cohort with 22 routinely measured hematological parameters, obtained from UPOD, which comprises clinically relevant data from all patients admitted to the University Medical Center Utrecht, including laboratory measurements. Hematology measurements were performed as part of clinical routine in EDTA blood on the Sapphire hematology analyzers (Abbott, Santa Clara, CA). It uses the multi-angle polarized scatter separation technique. Further details on the quantification of hematological parameters in UPOD have recently been published elsewhere [12].

**Clinical chemistry**

Clinical chemistry measurements, i.e. creatinine, total cholesterol, triglycerides, HDL-cholesterol and hs-CRP, were performed in Li-heparin plasma on clinical routine IVD analyzers (AU5800, Beckman Coulter, Brea, CA) at the central diagnostic laboratory of the UMC Utrecht according to international standards (ISO9001, ISO15189). LDL-cholesterol was calculated using the Friedewald equation; eGFR was calculated from creatinine levels according to the MDRD formula.

**Statistical analysis**

As for the derivation of the SRS, we truncated all continuous variables, including all hematological parameters, at the 1st and the 99th percentile to reduce the impact of outliers [9]. Using single imputation by additive regression, we imputed missing values for all variables included in the SRS (total n = 126; 0.2%). The variable with the highest percentage of missing values was hs-CRP (n = 75; 1.9%). To facilitate comparison between different hematological parameters, all values were scaled to SD units prior to analysis.

We first evaluated associations between each of the 22 hematological parameters and recurrent vascular events, using Cox proportional hazards modeling adjusted for all SRS variables [age, sex, diabetes mellitus, current smoking, systolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, hs-CRP, estimated glomerular filtration rate (eGFR), years since first vascular event, history of cerebrovascular disease, history of coronary artery disease, history of abdominal aortic aneurysm, history of peripheral artery disease]. Analogous to the SRS, hs-CRP was log_e-transformed and quadratic terms were added for age and eGFR.
Since none of hematological parameters showed a skewness >2, log_e-transformation was not applied. Hematological parameters were entered as quadratic polynomials if the addition of a quadratic term improved model fit, as indicated by the likelihood ratio test (p < 0.05). Accordingly, we added a quadratic term for hematocrit. The proportional hazards assumption was tested for each model using scaled Schoenfeld residuals. Associations between hematological parameters and outcome were adjusted for multiple testing. Since several of the 22 parameters were highly correlated (Figure A in S1 File), we estimated the effective number of independent tests for multiple testing correction using principal component analysis. The first 11 principal components explained over 95% of the variance in the hematology data, yielding a significance threshold of 0.05/11 = 0.0045.

We next evaluated the added predictive value of hematological parameters, significantly associated with outcome, by comparing different biomarker models to a reference model in terms of discrimination and reclassification. The reference model was constructed by fitting the SRS variables to our dataset. The single biomarker models included the SRS variables and one of the hematological parameters significantly associated with recurrent event risk. We additionally assessed the performance of multi-biomarker models that included combinations of hematological parameters. To evaluate discrimination, we calculated Harrell’s c for each model and compared c-statistics between each biomarker model and the reference model, using the jackknife approach proposed by Antolini et al [14]. Extending the area under the receiver operating characteristic (ROC) curve to censored outcomes, Harrell’s c measures the ability of a risk prediction model to discriminate individuals with a target events from event-free individuals. Reclassification was assessed by continuous net reclassification improvement (cNRI), as implemented in the nricens R package (https://cran.r-project.org/web/packages/nricens/index.html), which computes NRI for censored survival data. Confidence intervals for NRI were computed by bootstrapping. To obtain robust reclassification indices, we assessed cNRI at 7 years, given a median follow-up of 4.6 years (IQR: 2.5–6.9 years). 7 years also corresponds to the follow-up period for which the SRS was initially calibrated before risk estimates were extrapolated to 10-year risk predictions [9]. Due to the absence of established categories for the 7-year risk of recurrent vascular events, we did not assess categorical NRI.

Results

3,922 patients with manifest vascular disease enrolled in the SMART cohort were included in this study. Baseline characteristics of the study population are summarized in Table 1. During a median follow-up of 4.6 years (IQR: 2.5–6.9 years), 310 recurrent vascular events occurred. In contrast to Dorresteijn et al. [9], we only included patients recruited from 2005 onwards. Compared to this study, we observed lower event rates (1.7% vs. 2.6%), most likely reflecting improved secondary prevention therapies. In line with this, the proportion of patients treated with statins was higher in our study. Table 2 shows baseline values of all 22 hematological parameters stratified by event status.

First, we studied associations between hematological parameters and secondary vascular outcomes. Table A in S1 File displays unadjusted and adjusted hazard ratios (HRs) for all hematological parameters. HRs for all SRS variables (reference model) are shown in Table B in S1 File. Since most hematological parameters are directly or indirectly related to immunological processes, we assessed whether these associations were independent of hs-CRP. The addition of hs-CRP particularly attenuated effect estimates for white blood cell count, neutrophil count, monocyte count and neutrophil % (Fig B in S1 File). Four parameters remained significantly associated with vascular events after adjustment for the SRS variables (Fig 1). Lymphocyte % showed a negative association with the recurrent vascular events (HR in SD units: 0.80
Table 1. Baseline characteristics.

|                      | All (N = 3922) | No vascular event (N = 3612) | Vascular event (N = 310) |
|----------------------|---------------|----------------------------|--------------------------|
| Age, years           | 61 (54–68)    | 61 (54–67)                 | 64 (56–71)               |
| Male sex             | 2850 (73)     | 2610 (72)                  | 240 (77)                 |
| Type of vascular disease |              |                            |                          |
| Cerebrovascular disease | 1125 (29)    | 1032 (29)                  | 93 (30)                  |
| Coronary artery disease | 2588 (66)    | 2373 (66)                  | 215 (69)                 |
| Peripheral artery disease | 531 (14)     | 481 (13)                   | 50 (16)                  |
| Abdominal aortic aneurysm | 236 (6)      | 213 (6)                    | 23 (7)                   |
| Years since first vascular event |              |                            |                          |
| less than 1 year     | 2283 (60)     | 2140 (61)                  | 143 (48)                 |
| 1–2 years            | 389 (10)      | 363 (10)                   | 26 (9)                   |
| over 2 years         | 1110 (29)     | 980 (28)                   | 130 (44)                 |
| Current smoking      | 1060 (27)     | 954 (27)                   | 106 (34)                 |
| Diabetes mellitus    | 704 (18)      | 628 (17)                   | 76 (25)                  |
| Systolic blood pressure, mm Hg | 136 (124–149) | 135 (124–149)              | 140 (129–155)            |
| Diastolic blood pressure, mm Hg | 80 (73–88)     | 80 (74–88)                 | 81 (73–90)               |
| eGFR, ml/min/1.73 m² | 77 (66–88)    | 77 (67–88)                 | 70 (60–84)               |
| Total cholesterol, mmol/l | 4.3 (3.7–5.1) | 4.3 (3.7–5.1)              | 4.3 (3.7–5.1)            |
| LDL cholesterol, mmol/l | 2.4 (1.9–3.0) | 2.4 (1.9–3.0)              | 2.4 (1.9–3.1)            |
| HDL cholesterol, mmol/l | 1.2 (1.0–1.4) | 1.2 (1.0–1.4)              | 1.1 (1.0–1.4)            |
| Triglycerides, mmol/l | 1.2 (0.9–1.8) | 1.2 (0.9–1.8)              | 1.3 (0.9–1.9)            |
| hs-CRP, nmol/l       | 16 (8–36)     | 15 (8–34)                  | 26 (12–62)               |
| Medication           |               |                            |                          |
| Lipid-lowering drugs | 3140 (80)     | 2888 (80)                  | 252 (81)                 |
| Blood pressure-lowering drugs | 3086 (79) | 2829 (78)                  | 257 (83)                 |
| Glucose-lowering drugs | 560 (14)     | 497 (14)                   | 63 (20)                  |
| Antithrombotic drugs | 3493 (89)     | 3206 (89)                  | 287 (93)                 |

Discrete variables are expressed as count (%), continuous variables as median (IQR). Type of vascular disease is not mutually exclusive as patients may have experienced several manifestations of vascular disease. eGFR: estimated glomerular filtration rate (see [9]); HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; IQR: inter-quartile range; LDL: low-density lipoprotein.

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[95% CI: 0.71, 0.91]), whereas neutrophil count (HR in SD units: 1.19 [1.06, 1.33]), neutrophil % (HR in SD units: 1.22 [1.08, 1.37]), and RDW (HR in SD units: 1.16 [1.05, 1.28]) were positively associated with recurrent vascular events.

To assess discrimination and continuous reclassification, we next added each of the four hematological parameters that were independently associated with recurrent event risk to a reference model composed of the SRS variables (Table 3). We observed the largest cNRI for lymphocyte %. For events, this parameter improved continuous reclassification by 13.6%, for non-events by 3.8%, yielding a cNRI of 17.4% [95% CI: 2.1, 32.1%]. Additionally, lymphocyte % improved discrimination (c-statistic) by 0.0110 [95% CI: 0.0004, 0.0216]. We also tested whether lymphocyte % combined with other parameters further improved the predictive performance of the SRS. Neutrophil % was not included into a multi-biomarker model because this parameter was highly correlated with lymphocyte % (r = -0.92). Lymphocyte % and neutrophil count improved cNRI by 22.2% [3.2, 33.4%]. The increase in c-statistic was 0.0112 [0.0004, 0.220], which was comparable to that achieved by lymphocyte % alone. For lymphocyte % and RDW combined, the cNRI was 18.7% [3.3, 31.9%], the improvement in c-statistic
was 0.016 [0.004, 0.028]. With a cNRI of 17.2% [4.1, 32.8%], all three parameters yielded a lower reclassification improvement than the combination of lymphocyte % and neutrophil count. Lymphocyte % in combination with RDW improved discrimination with an increase in c-statistic of 0.016 [0.004, 0.028]. Fig 2 illustrates the change in predicted risk for different biomarker models, stratified by event status. While lymphocyte % alone and the combination of lymphocyte % and neutrophil count showed the largest continuous reclassification improvement (Table 3) for events, risk estimates increased only modestly in patients who experienced an event. Lymphocyte % and RDW combined predominantly increased risk estimates for events in the higher risk range.

### Discussion

In this study, we evaluated the incremental predictive value of routinely measured hematological parameters for the prediction of recurrent vascular events in patients with established vascular disease. We first investigated associations between 22 parameters and recurrent event risk and then assessed whether parameters associated with outcome improved risk prediction. Out of the four parameters significantly associated with outcome, lymphocyte % showed the largest cNRI when individually added to the SRS. Overall, the combination of lymphocyte % and neutrophil count yielded the largest cNRI compared to the SRS, but only modestly improved discrimination (c-statistic) and risk estimates for patients who experienced an event during follow-up.
Lymphocytes have been implicated in the modulation of inflammatory processes at distinct stages of atherogenesis [15]. Numerous observational studies in patients with coronary artery disease have reported associations of low absolute and relative lymphocyte levels with poor clinical outcomes.

![Graph showing hematological parameters](https://doi.org/10.1371/journal.pone.0202682.g001)

**Table 3. Predictive performance of hematological parameters.**

| Parameter | Change in c-statistic [95% CI] | with event | without event | Net [95% CI] |
|-----------|---------------------------------|-----------|---------------|-------------|
| Neutrophils | 0.006 [-0.002, 0.014] | -9.1 | 15.6 | 6.5 [-6.0, 22.7] |
| Neutrophil % | 0.008 [-0.002, 0.018] | 7.2 | 6.7 | 13.9 [-0.3, 27.7] |
| Lymphocyte % | 0.011 [0.000, 0.022] | 13.6 | 3.8 | 17.4 [2.1, 32.1] |
| RDW | 0.007 [-0.001, 0.015] | -11.3 | 25.0 | 13.6 [-1.9, 26.4] |
| Lymphocyte % + neutrophils | 0.011 [0.000, 0.022] | 14.8 | 7.4 | 22.2 [3.2, 33.4] |
| Lymphocyte % + RDW | 0.016 [0.004, 0.028] | 9.0 | 9.7 | 18.7 [3.3, 31.9] |
| Lymphocyte % + neutrophils + RDW | 0.016 [0.004, 0.028] | 5.1 | 12.0 | 17.2 [4.1, 32.8] |

First, hematological parameters significantly associated with outcome were individually added to a reference model composed of the SRS variables. For each single biomarker model (SRS + hematological parameter), we evaluated improvement in discrimination (c-statistic) and reclassification (NRI) compared to the reference model (SRS). We then assessed the predictive performance of multi-biomarker models comprising combinations of lymphocyte % and other hematological parameters. NRI: net reclassification improvement; RDW: red cell distribution width; SRS: SMART risk score.

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cardiovascular outcomes [12,16–21]. However, some studies found no link between absolute lymphocyte count and all-cause mortality in pre-existing coronary artery disease [22–24]. Consistent with a role of low lymphocyte levels in vascular disease progression, lymphocyte apoptosis is enhanced in myocardial infarction, but not in stable angina, indicating that low lymphocyte levels may specifically reflect inflammatory processes in advanced atherosclerosis (e.g. plaque rupture) [25]. In our study, however, lymphocyte % rather than absolute lymphocyte count was associated with recurrent vascular events. Accordingly, lymphocyte levels were
comparable between patients with and without a recurrent event during follow-up—unlike concentrations of other white blood cell types, such as neutrophils and monocytes (Table 2). Low lymphocyte % may thus reflect increased levels of other white blood cell types in patients at risk.

Besides lymphocyte %, both absolute and relative neutrophil count were independently associated with recurrent vascular risk without improving risk prediction when individually added to the SRS. The combination of lymphocyte % and absolute neutrophil count showed the largest cNRI of all models assessed, but only moderately increased risk estimates for events. The discrimination improvement with lymphocyte % and absolute neutrophil count was likewise limited with an increase in c-statistic equal to that achieved by lymphocyte % alone. The neutrophil to lymphocyte ratio has been widely studied as a marker of cardiovascular risk, suggesting that neutrophil levels are associated with poor prognosis of coronary and peripheral artery disease [26]. There is mounting evidence that neutrophils play an important role in early and advanced atherosclerosis by exacerbating endothelial dysfunction, recruiting monocytes to atherosclerotic lesions, promoting foam cell formation and by destabilizing atherosclerotic plaques [27].

RDW was also independently associated with clinical outcome. Several studies have linked increased RDW to poor outcomes in patients with coronary artery disease, stroke or peripheral artery disease [12,28–31]. RDW is a measure of the variation in erythrocyte volume. The mechanisms by which RDW relates to cardiovascular risk are unknown. Severe inflammation is associated with inhibition of erythrocyte maturation, which results in anisocytosis, suggesting that RDW reflects enhanced inflammation in atherosclerosis, potentially relevant to disease progression [32]. However, RDW did not improve risk prediction and, when combined with lymphocyte %, yielded a cNRI comparable to that achieved by lymphocyte % alone. Moreover, RDW and lymphocyte % predominantly increased risk estimates for events in the higher risk range. Since patients with a high SRS would already be eligible for increased surveillance and more extensive treatment, the added value of RDW for clinical risk prediction is limited.

In the unadjusted analysis, total white blood cell count and monocyte count were strongly associated with recurrent events. However, adjustment for all SRS variables attenuated effect estimates for both parameters, especially due to the inflammatory marker hs-CRP (Fig B in S1 File). In vitro findings suggest that CRP interacts with monocytes to enhance inflammation in acute coronary syndrome [33]. Thus, hs-CRP and monocytes may share a common pathophysiological pathway, whereas other hematological parameters may reflect inflammatory processes that do not, or to a lesser extent, involve CRP. Overall, our findings lend further support to the inflammatory hypothesis of atherothrombosis and add to recent clinical trial data suggesting that anti-inflammatory therapy reduces cardiovascular risk in secondary prevention [34].

Hematological parameters are routinely measured in many hospitals and do not require expensive equipment for analysis, underscoring their clinical potential. In our study, lymphocyte % alone and combined with other hematological parameters yielded the largest cNRI. However, these models only marginally improved discrimination and absolute risk estimates for events. Thus, it remains to be determined whether the incorporation of hematological parameters into risk prediction algorithms would influence clinical decision making in secondary prevention. Since many clinical and demographic characteristics are not assessed systematically in clinical routine, it is often not possible to calculate clinical scores, such as the SRS. Routine hematology testing may be combined with other emerging biomarker technologies suitable for clinical laboratory use to construct biomarker risk scores that do not depend on the availability of clinical information. Such biomarker-based scores could routinely be
computed by clinical chemistry laboratories, facilitating the implementation of risk assessment tools for secondary prevention in clinical practice. Besides adding hematological parameters to established clinical scores, future studies also evaluate their predictive value in combination with other biomarkers.

Moreover, the ability of hematological parameters to predict recurrent vascular risk may vary between different manifestations of vascular disease, such as myocardial infarction and ischemic stroke. Since hematological parameters were not available from all SMART patients, the sample size of our study population was limited. As a result, we could not perform stratified analyses for different vascular disease groups. Therefore, further research is required to corroborate our findings in larger cohorts and establish the predictive value of hematological parameters for different manifestations of vascular disease.

In conclusion, we identified several hematological parameters that were independently associated recurrent vascular event in patients with vascular disease. When added to a model comprising the SRS variables, lymphocyte % alone and in combination with other hematological parameters, especially with neutrophil count, improved risk prediction, but only modestly increased risk estimates for patients who experienced a recurrent vascular event.

Supporting information
S1 File. Supporting information.
(PDF)

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