Hematological Parameters Outperform Plasma Markers in Predicting Long-Term Mortality after Coronary Angiography

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Acknowledgements: We sincerely thank Miss Jonne Hos for her excellent contribution to the UCORBIO cohort.

There are no relationships with the industry to declare.

Abstract

High-sensitivity troponin I (hsTnI) and N-terminal pro-brain natriuretic peptide (NTpro-BNP) are predictors of coronary artery disease. Recently, routine hematological parameters emerged as mortality predictors. We examined the predictive value of hematological parameters (from the Utrecht Patient Oriented Database; UPOD) and hsTnI and NTpro-BNP for mortality in a coronary angiography population (Utrecht Coronary Biobank n=1,913). Using Cox regression, receiver operating characteristics, integrated discrimination improvement (IDI) and continuous net reclassification improvement (cNRI) analysis, we compared the predictive properties of hematological parameters with hsTnI and NTpro-BNP for mortality. During a median follow-up duration of 1.8 years, 77 deaths occurred. A panel of 7 hematological parameters (leukocyte count; reticulocyte mean corpuscular hemoglobin concentration; red blood cell (RBC) green (FL1) fluorescence; %neutrophils; %large (>120fL) RBCs, %monocytes and coefficient of variation of neutrophil complexity) was highly predictive. Added to clinical characteristics, hematological parameters (area under the curve [AUC]:0.856, p<0.001, IDI:0.07, p<0.001, cNRI:0.37, p<0.001) were better predictors than hsTnI (AUC:0.818) or NTpro-BNP (AUC:0.834) alone or combined (AUC:0.834). Hematological parameters may provide mortality risk information following coronary angiography and may be superior to hsTnI and/or NTpro-BNP.

Keywords: coronary artery disease, biomarkers, mortality.

Introduction

High-sensitivity troponin I[1] (hsTnI) and NT pro-B-natriuretic peptide[2] (NTpro-BNP) constitute the current clinical gold-standard biomarkers for diagnosis and prognosis in acute myocardial infarction,[3] stable heart failure,[4] elective coronary angiography,[5] stable and unstable coronary artery disease (CAD)[6,7] and percutaneous coronary intervention[8] (PCI) patients.

Recently, a different and easily accessible type of biomarker has emerged. Blood cell characteristics (counts and percentages), e.g. from leukocytes[9,10] and red blood cells (RBCs),[11] harbor prognostic information in diverse patient populations. Such hematological parameters are widely available and measured on a routine basis. Modern automated hematology analyzers automatically perform a whole blood cell count irrespective of the clinical request. While not routinely reported to the physician, the unrequested parameters can be extracted and stored for future reference. In the University Medical Center in Utrecht, blood cell differentiation data from the Abbott Sapphire[12] hematology analyzer have been stored in the Utrecht Patient Oriented Database (UPOD)[13] for research purposes. The measured parameters consist of cell counts, cell sizes and other cell properties such as cell complexity and granularity.

As precise estimation mortality risk in CAD patients is key for a patient-specific treatment policy and for accurate patient information, we compared the predictive power of routine hematological parameters with the current clinical standard, hsTnI and NTpro-BNP, for prediction of mortality during two years of follow-up in CAD patients undergoing coronary angiography.

Methods

Study population

In this study we analyzed data from the Utrecht Coronary Biobank (UCORBIO) cohort (registered as an observational study at clinicaltrials.gov, identifier: NCT02304744), an observational cohort study of coronary angiography patients in the University Medical Center in Utrecht, the Netherlands. From October 2011 to December 2014, a total of 2,591 patients were enrolled. For the current study, we selected adult (>18 years) patients presenting with

myocardial infarction (either ST-Segment Elevation Myocardial Infarction or Non-ST-Segment Elevation Myocardial Infarction), chest pain without release of cardiac enzymes (stable or unstable angina), dyspnea on exertion, silent ischemia or screening for non-cardiac surgery with complete case information (n=1,913). Patients with other indications for coronary angiography (coronary anomalies, screening for cardiac surgery or follow-up after heart transplantation) were excluded.

Ethics, consent and permissions

All patients provided written informed consent and the study conforms to the Declaration of Helsinki. The institutional review board of the University Medical Centre Utrecht approved of this study (registration number 11-183).

Data collection

Data collection in the UCORBIO cohort has been described before.[14] In summary, standardized electronic case report forms were completed at baseline containing age, sex, cardiovascular risk factors, indication for angiography, medication use, angiographic findings and eventual treatment of CAD. The angiographic findings were categorized into 4 groups by the treating interventional cardiologist: no CAD, minor CAD (wall irregularities, <50% stenosis), single vessel disease (one vessel with >50% stenosis[15]) and multi-vessel disease (2 or 3 vessels with >50% stenosis).

Plasma Biomarkers

Plasma biomarkers (hsTnI and NTpro-BNP) were measured in thawed EDTA plasma, which had been drawn directly prior to coronary angiography from the arterial sheath, before heparinization and immediately stored at -80°C. hsTnI was measured using the *STAT* High Sensitive Troponin-I assay on the ARCHITECT *i*2000 analyzer (Abbott Park, Illinois, USA). NTpro-BNP was measured using a semi-automated ELISA robot (Freedom EVO, Tecan, Switzerland, antibodies: 15C4 and biotinylated 13G12, Hi-test Finland).

Hematological parameters

Hematological parameters were obtained through complete blood count analysis at inclusion from the same blood sample as the plasma biomarkers. Fifty-six routinely measured RBC, leukocyte and platelet parameters from the UPOD database were initially taken into consideration in this study.[13] All hematological parameters were measured using the Cell-Dyn

Sapphire[16] hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). This analyzer is equipped with an integrated 488-nm blue diode laser and uses spectrophotometry, electrical impedance, laser light scattering (multi-angle polarized scatter separation), and 3-color fluorescent technology to measure morphological parameters of leukocytes, RBCs and platelets for classification and enumeration. The morphological parameters entail the following 5 optical scatter signals for leukocytes: cell size (0˚ scatter, axial light loss), cell complexity and granularity (7˚ scatter, intermediate angle scatter (IAS)), nuclear lobularity (90˚ scatter, polarized side scatter (PSS)), depolarization (90˚ depolarized side scatter (DSS)) and viability (red fluorescence (FL-3), 630 ± 30 nm). Platelets are analyzed using two optical scatter signals: IAS scatter (7˚, cell size) and PSS scatter (90˚, granularity; internal structure). RBC parameters are measured or calculated based on impedance measurements. Reticulocytes are optically measured using IAS scatter (7˚, cell size) and FL-1 fluorescence (RNA content). Throughout this paper, all hematological parameters are reported as multitudes of their standard deviation (SD) to facilitate comparison of effect sizes between parameters as their absolute values vary strongly in their order of magnitude.

Statistical Analysis

Baseline characteristics are reported as means and standard deviations for continuous variables and percentages for categorical variables, both, for the entire cohort and for survivors and non-survivors separately.

First, we constructed a clinical risk prediction model. Covariates for this model were selected using a backward stepwise Cox regression model for all-cause mortality, which and comprised: age, sex, diabetes, hypercholesterolemia, smoking status, indication for angiography, angiographic CAD severity, history of PCI, history of acute coronary syndrome (ACS), kidney failure and treatment following angiography. The model with the lowest Akaike information criterion was selected. Assumptions for Cox regression were checked and satisfied.

To determine a predictive panel of hematological parameters we first evaluated mutual correlation of the parameters (total n=56) by means of hierarchically clustered heatmap analysis, grouping closely related parameters in a cluster. From each cluster of collinear parameters (spearman's R>0.6 or <-0.6) the parameter with the strongest relation

with all-cause mortality was selected for further analysis. The remaining parameters (n=34, supplemental table 1) were entered in a backward stepwise Cox regression model. Subsequently, the top 10 significant parameters were added to the clinical model (which was coerced to stay in the model) and backward stepwise Cox regression was performed again to determine the final panel of hematological parameters.

Receiver operating characteristics (ROC) analysis was performed to assess the prognostic value of hsTnI, NTpro-BNP and the panel of hematological parameters in addition to the clinical model. The clinical model was entered as a linear predictor to stabilize its predictive value. Next, we evaluated the prognostic value of adding hsTnI, NTpro-BNP or both to the panel of hematological parameters (all on top of the clinical model).

For visualization of the predictive value of the panel of hematological parameters, a linear predictor was constructed and the predicted risk based on this linear predictor was divided into quartiles. Survival, adjusted for the clinical parameters was subsequently plotted for these quartiles.

Internal validation of the predictive properties of the hematological parameters was performed by means of post-estimation parameterwise shrinkage[17] using the jackknife method (repeating the analysis leaving out 1 observation at a time). For this purpose we used the “shrink” package[18] for R. Also, optimism-adjusted AUCs were calculated using a bootstrapping method.

Furthermore, continuous net reclassification improvement (cNRI) and integrated discrimination improvement (IDI) measures for the abovementioned comparisons were calculated using the “survIDINRI” package[19] to assess risk prediction improvement. The cNRI corresponds to the percentage of patients that is correctly reclassified by the addition of a new parameter to the previous Cox model and is calculated by adding the percentage of deceased patients who appropriately had higher predicted risk in the new model to the percentage of alive patients who appropriately had a lower predicted risk in the new model.[20] Continuous NRI was deemed preferable over categorical NRI due to the lack of established meaningful risk categories in secondary risk prediction.[21]

The IDI corresponds to the absolute change in predicted risk between the old and the new model. It is calculated by subtracting the difference in predicted risk between deceased

and alive patients in the old model from the difference in predicted risk between deceased and alive patients in the new model.[22] Additionally, we created optimism-adjusted calibration plots using bootstrapping (repeating the analysis using random resampling with replacement, n=40), to assess the model fit.

All statistical analyses were performed using Rstudio and the R software package (version 3.1.2, Vienna, Austria).[23] A two-sided p <0.05 was considered significant.

Results

Patient characteristics

During a median follow-up duration of 1.8 years, 77 deaths occurred; 29 of which from cardiovascular events. Patient characteristics are shown in table 1, stratified by mortality status during follow-up. Patients who died during the follow-up period were older (73 *vs* 63 years, p<0.001) and more frequently diabetic than survivors (38 *vs* 22%, p=0.002). Patients who died during follow-up more often had a history of coronary artery bypass grafting surgery, peripheral arterial disease, kidney failure and impaired left ventricular function. Angiotensin-converting enzyme inhibitor (48 *vs* 34%, p=0.018) and diuretic use was significantly more common in deceased patients (55.8 *vs* 27.4%, p<0.001). Median hsTnI levels (22.3 *vs* 7.1 ng/mL, p<0.001) and NTpro-BNP levels (260.5 *vs* 83.0 pmol/L, p<0.001) were significantly higher in deceased patients. The multivariable adjusted HR of hsTnI and NTpro-BNP for mortality during follow-up were 1.00 [0.93-1.08] per 1000 ng/mL increase, p=0.945 and 1.27 [1.14-1.42], p<0.001 per 1000 pmol/L increase, respectively.

Hematological parameters

Backward Cox regression was performed as described above, providing a panel of 7 hematological parameters. The levels of the hematological parameters for deceased and alive patients and multivariable adjusted hazard ratios (HRs) are displayed in table 2. When adjusted for clinical characteristics and the other selected hematological parameters the hazard ratios (HRs) were as follows: leukocyte count: HR 1.25 [1.12-1.39], p<0.001; reticulocyte mean corpuscular hemoglobin concentration (MCHCr): HR 0.65 [0.50-0.86], p=0.003; RBC green (FL1) fluorescence: HR 1.51 [1.15-1.97], p=0.003; % neutrophils: HR 1.37 [1.07-1.75], p=0.012; % large (>120fL) RBCs: HR 1.17 [1.03-1.34], p=0.019; %

monocytes: HR 1.28 [1.04-1.59], p=0.023 and coefficient of variation (CV) of neutrophil complexity: HR 1.31 [1.03-1.67], p=0.026.

All-cause mortality prediction

We first evaluated the additive predictive value of hsTnI, NTpro-BNP and hematological parameters to a clinical model (table 3 top) for the prediction of all-cause mortality. hsTnI did not improve prediction of mortality in addition to the clinical model (AUC-increase, IDI and cNRI all non-significant). NTpro-BNP on top of the clinical model significantly improved prediction (AUC 0.834 *vs* 0.818, p=0.019) (Figure 1A) and discrimination (IDI 0.02 [0.00-0.06], p=0.040), but not reclassification (cNRI 0.03 [-0.14-0.22], p=0.625). The combination of hsTnI and NTpro-BNP also improved the AUC from 0.818 to 0.834, p=0.016 compared with the clinical model alone, but the IDI and cNRI were both non-significant.

The addition of hematological parameters to the baseline clinical model (AUC 0.818) significantly improved discrimination (IDI 0.07 [0.03-0.14], p<0.001) and reclassification (cNRI 0.37 [0.19-0.49], p<0.001). The AUC increased to 0.856, p<0.001.

We then assessed whether hsTnI, NTpro-BNP or their combination could improve prediction in addition to the clinical model enriched by the hematological parameters (table 3 bottom, Figure 1B). While the AUC increased slightly, albeit non-significantly upon addition of NTpro-BNP (AUC 0.863 *vs* 0.856, p=0.061), only the combination with hsTnI significantly improved prediction (AUC 0.865, p=0.049). Neither hsTnI, NTpro-BNP nor their combination could significantly improve discrimination or reclassification (IDIs and cNRIs all non-significant, table 3).

Bootstrapped calibration plots were created (supplemental figure 1) in order to assess goodness-of-fit. Addition of hematological parameters to the clinical model significantly increased the R^2 (from 0.119 to 0.177, p<0.001), reflecting a better fit. When hsTnI was added to the model, it did not further improve the model fit. However, addition of NTpro-BNP slightly increased model fit (R^2 increased to 0.190, p=0.003). Optimism-adjusted AUCs provided similar results compared with the AUCs derived from our original models (supplementary figure 3).

Internal Validation

Models based on a single dataset tend to result in overfitting and overoptimistic estimates. Therefore, our model was internally validated by means of post-estimation shrinkage. The predicted all-cause mortality risk based on the initial model including hematological parameters was grouped into quartiles (Q1 to Q4). Adjusted survival curves are shown in Figure 2. HR for Q3 *vs* Q1 and Q4 *vs* Q1 were 6.6 [2.0-21.9], p=0.002 and 9.6 [3.0-31.2], p<0.001, respectively. After shrinkage, the HR for Q2 *vs* Q1 remained non-significant. HR for Q3 *vs* Q1 and Q4 *vs* Q1 were 3.2 [2.0-5.2], p=0.010 and 4.8 [3.2-7.0], p<0.001, respectively.

Discussion

Our study shows superiority of hematological parameters over the current clinical standard hsTnI and NTpro-BNP for mortality prediction in CAD patients undergoing coronary angiography. The resulting panel of parameters comprises leukocyte and RBC characteristics. Both, leukocyte and RBC characteristics have previously been reported to serve as strong predictors of mortality in various patient groups.

In cardiovascular disease patients, leukocyte characteristics have been tested and compared with the established marker high-sensitivity C-reactive protein (hsCRP). In stable CAD patients undergoing coronary angiography, neutrophil count was superior to hsCRP in predicting cardiovascular mortality.[9] In our study, we also included RBC and platelet characteristics as potential predictors of mortality. High platelet reactivity is an independent predictor of future adverse events in myocardial infarction patients and thus seemed to be a logical candidate for testing.[24] Unexpectedly, none of the platelet characteristics (plateletcrit, mean platelet volume, platelet distribution width, CV of platelet granularity and reticulated platelet count) in UPOD added any predictive value to the clinical model in our study; mean platelet volume did however predict mortality in univariable analysis in agreement with the literature.[25] The reasons can be manifold. Our study population mainly consists of stable CAD patients, which have a lower risk of acute fatal thrombotic events due to high platelet reactivity than myocardial infarction patients. Hence, the role of platelets in this cohort might be less relevant. More likely though, the predictive value of platelet characteristics is also reflected by other hematological parameters in our multivariable model, rendering it non-significant. Another possibility is that high platelet reactivity is not reflected by their morphology as measured by the hematology analyzers, supporting the need for separate platelet activation testing in high-risk patients. Finally, though our analyses did not investigate such effects, the lack of platelet markers might be due to platelet inhibitors given to the vast majority of patients included in this study.

In contrast, both leukocyte and RBC parameters provided additional predictive value. Monocytes have long been known for their causal role in plaque initiation, progression and destabilization.[26] As previously shown, their numbers correlate with the presence of

cardiovascular disease, higher IL-6 levels and predict all-cause and cardiovascular mortality.[27,28]

Recent studies have shown the direct involvement of neutrophils in cardiovascular disease. Neutrophil depletion significantly reduced plaque formation in mice.[29] Furthermore, neutrophils can be found in high numbers in coronary artery autopsy specimens from patients who died of myocardial infarction.[30] In addition to neutrophil numbers, we found additional predictive value of the variation in neutrophil complexity. This can be regarded as an indication of a 'left shift' or neutrophil activation as frequently seen in acute infections.[31] Morphological changes of neutrophils have also recently been described to correlate with cardiac function after acute myocardial infarction in a porcine model.[32] The observed 'left shift' could thus reflect subclinical chronic inflammation due to prevailing coronary atherosclerosis or subacute myocardial ischemia. This is supported by the interaction between neutrophil numbers and morphology on one side and atherosclerosis and ischemia (or vice versa[29]) on the other. The exact mechanisms relating these neutrophil characteristics to increased mortality risk however remain to be elucidated.

The role of RBCs in atherosclerosis is less clear. Nevertheless, they harbor significant predictive value in various diseases. In particular RBC distribution width[33–35] (RDW), which reflects the variation in RBC volume, has been proposed as a powerful risk indicator of mortality.[11] RBC volume is inversely related with RBC age; young cells are largest, senescent cells are smaller.[36] RBC characteristics, particularly the % of large (i.e. young) RBCs that independently predicted mortality in our study concurred with a higher proportion of young erythrocytes (closely related to RDW, $r= 0.21$, $p<0.001$). RBC green (FL1) fluorescence, which was associated with worse survival in our study reflects the amount of residual RNA in young erythrocytes and reticulocytes. Thus, it could reflect a higher percentage of young RBC, but its clinical relevance remains unclear.

Together with the MCHCr, markers of an immature RBC population with a low reticulocyte hemoglobin concentration are thus related to a higher risk of mortality in coronary angiography patients.

To our surprise, we did not find any predictive value of hsTnI in our cohort. High-sensitivity troponin T (hsTnT) has been reported by several other groups as a potential

predictor of mortality, for example in stroke,[37] after cardiac surgery[38] and after elective coronary angiography.[5] Possibly, this finding is due to differential prognostic properties of hsTnI and hsTnT, as reported by de Antonio et al.[39]

High BNP levels are predictive of adverse events in a population of stable CAD patients[6] and NTpro-BNP has been shown to predict mortality in an unstable CAD population.[40] Our study is in line with others, showing that the predictive value of NTpro-BNP for mortality is superior to hsTnI.[41]

Nevertheless, hematological parameters in our study outperformed both NTpro-BNP and hsTnI for prediction of mortality. Apparently, blood cell characteristics provide more prognostic information than the cardiac-specific biomarkers hsTnI and/or NTpro-BNP.

The hematological parameters included in our panel are of leukocyte (total leukocyte count, neutrophil %, monocyte % and neutrophil complexity CV) and RBC origin (MCHCr, RBC green (FL1) fluorescence and % large RBCs). Leukocyte[42] and neutrophil counts[43] have previously been described as prognostic markers for mortality in a population-based cohort. Neutrophil count was not included in our analysis due to its collinearity ($r=0.68$, $p<0.001$) with neutrophil %, which was included as it had a stronger association with mortality.

The reason that blood cells convey more accurate prognostic information than cardiac specific biomarkers could be related to the organ-specificity of hsTnI and NTpro-BNP. The end-point in our study was all-cause mortality, meaning that not all deaths were due to cardiovascular disease or its consequences per se. Hematological parameters could provide a general whole-body overview of an individual's health status and subsequent prognosis. Therefore, we performed an additional analysis for cardiovascular death only. Again, hematological parameters outperformed hsTnI and NTpro-BNP (supplemental figure 2). However, the number of cardiovascular deaths was low (n = 29) and these data should thus be interpreted with caution.

In this manuscript the clinical parameters were analyzed as a linear predictor, meaning that the predictive value of these parameters was kept fixed when adding new hematological parameters. This might be a limitation to this study.

Several groups have investigated the relation between statin use and hematological parameters.[44,45] In our study our results did not change when adding statin use to the

prediction model, also there was no interaction between statin use and the predictive value of hematological parameters ($p_{interaction}$ 0.9).

The past few years many hematological parameters and ratios have been proposed for prediction in cardiovascular diseases, such as the leukocyte count,[46] monocyte-to-lymphocyte ratio,[10] neutrophil-to-lymphocyte ratio[47] and so on. While it is unclear which hematological parameter will prove to be the best predictor, it logically makes sense that a panel of several hematological parameters is more accurate than a single parameter or ratio between two, as proposed in this manuscript. External validation of our findings remains indicated.

Conclusions

Hematological parameters outperform the established biomarkers hsTnI or NTpro-BNP alone or in combination in predicting all-cause mortality after coronary angiography. Hence, readily available hematological parameters may provide a useful tool to improve current risk prediction algorithms (possibly integrated within the hematology analyzer linked to the electronic patient records) for all-cause mortality in coronary angiography patients. While we put every effort in validating our model internally, external validation is warranted to investigate the clinical use of these parameters and their extension to other cardiovascular disease patient groups.

Disclosures

None.

Data access

Access to the data used to perform this research can be requested form the corresponding author.

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Figure 1. ROC plots of hematological parameters, hsTnI and NTpro-BNP; A) in addition to a clinical model for the prediction of all-cause mortality, B) in addition to a clinical model plus hematological parameters for the prediction of all-cause mortality

A) ROC plots of the clinical model and of the clinical model extended with hsTnI, NTpro-BNP (or both) and hematological parameters for the prediction of all-cause mortality during 2 years of follow-up. B) ROC plots of the clinical model plus hematological parameters and of that model extended with hsTnI, NTpro-BNP or both for the prediction of all-cause mortality during 2 years of follow-up. “Clin + H” stands for clinical model plus hematological parameters.

Abbreviations: ROC = receiver operating characteristics, hsTnI = high-sensitivity troponin I, NTpro-BNP = N-terminal pro-brain natriuretic peptide.

Figure 2. Survival plot showing survival by quartiles of predicted risk based on hematological parameters, adjusted for clinical characteristics

The predicted risk of all-cause mortality based on hematological parameters, was grouped into 4 quartiles (Q1 to Q4). See methods section for more detailed explanation. The multivariable adjusted survival in these quartiles was plotted using Cox regression analysis. The HR for Q2 *vs* Q1 was not significant, for Q3 *vs* Q1 it was 6.5 [2.0-21.8], p=0.002; the HR for Q4 *vs* Q1 was 11.8 [3.6-38.1], p<0.001. Abbreviations: HR = hazard ratio.

Supplemental figure 1. Calibration plots of prediction models

The predicted *vs* the observed risk is shown for the clinical model, the clinical model plus hematology, the clinical model plus hematology and hsTnI and the clinical model plus hematology and hsTnI and NTpro-BNP. The model fit is significantly improved by the addition of hematological parameters. Addition of hsTnI results in no improvement. Addition of NTpro-BNP however does slightly improve model fit further. *p-value from Wald-test comparing the Cox model to the previous one (without the added marker in question).

Abbreviations: hsTnI = high-sensitivity troponin I, NTpro-BNP = N-terminal pro-brain natriuretic peptide.

Supplemental figure 2. ROC plots of hematological parameters, hsTnI and NTpro-BNP in addition to a clinical model for the prediction of cardiovascular mortality

ROC plots of the clinical model and of the clinical model extended with hsTnI, NTpro-BNP (or both) and hematological parameters for the prediction of cardiovascular mortality during 2 years of follow-up. Abbreviations: ROC = receiver operating characteristics, hsTnI = high-sensitivity troponin I, NTpro-BNP = N-terminal pro-brain natriuretic peptide.

Supplemental figure 3. Optimism-adjusted AUCs for the models used in this manuscript.

These adjusted AUCs were calculated using a bootstrapping approach (n=100). Original and adjusted AUCs are shown in the red (adjusted AUC) and blue lines (original AUC). Abbreviations: AUC = area under the curve (from receiver operating characteristics analysis).

Table 1. Baseline characteristics of UCORBIO patients.

	Overall	Alive	Deceased	p
n	1913	1836	77	
Age, mean (sd)	63.7 (10.9)	63.3 (10.8)	72.5 (8.7)	<0.001
Sex (%)	73.9	73.9	72.7	0.921
BMI, mean (sd)	27.2 (4.5)	27.2 (4.5)	26.6 (4.8)	0.281
Diabetes (%)	22.7	22.1	37.7	0.002
Hypertension (%)	59.0	58.8	63.6	0.464
Hypercholesterolemia (%)	48.0	48.4	39.0	0.133
Smoking (%)				0.068
Active smoker	25.6	25.9	19.5	
Ex-smoker	26.5	26.0	37.7	
Non smoker	47.9	48.1	42.9	
History of ACS (%)	30.6	30.3	37.7	0.215
History of PCI (%)	27.7	27.8	23.4	0.468
History of CABG (%)	10.5	10.1	18.2	0.038
History of CVA (%)	10.5	10.2	16.9	0.091
History of PAD (%)	11.7	11.3	20.8	0.018
Kidney failure (%)	2.8	2.5	10.4	<0.001
EF (%)				<0.001
Normal	57.0	58.2	32.4	
Mildly impaired	23.2	23.1	25.0	
Impaired	12.1	11.7	20.6	
Poor	7.6	6.9	22.1	
Aspirin (%)	58.1	58.0	59.7	0.859
Clopidogrel (%)	20.8	20.9	18.2	0.658
Beta-blocker (%)	54.5	54.3	59.7	0.409
ACE inhibitor (%)	34.8	34.3	48.1	0.018
Statin (%)	61.6	61.5	63.6	0.802
Diuretic (%)	28.6	27.4	55.8	<0.001
Coronary Angiography				
Indication (%)				0.222
Stable CAD	56.5	56.5	55.8	
UAP	9.7	9.8	6.5	
Infarction	28.4	28.4	27.3	
Other	5.5	5.3	10.4	
Severity of CAD (%)				0.566
No CAD	6.3	6.3	6.5	
Minor CAD	15.2	15.0	19.5	
Single vessel disease	33.7	33.9	27.3	
Multi vessel disease	44.9	44.8	46.8	
Procedure (%)				0.613
Conservative	31.3	31.1	36.4	
PCI	62.6	62.8	58.4	
CABG	6.1	6.1	5.2	
hsTnI (ng/mL, median [IQR])	7.4 [3.7, 30.1]	7.1 [3.6, 27.4]	22.3 [5.1, 65.9]	<0.001
NT proBNP (pmol/L, median [IQR])	86.4 [33.4, 210.5]	83.0 [32.6, 199.8]	260.5 [89.5, 598.4]	<0.001
FU in years (median [IQR])	1.8 [1.0, 2.6]	1.7 [1.0, 2.5]	2.2 [1.8, 2.9]	<0.001

Abbreviations: UCORBIO = Utrecht Coronary Biobank, BMI = body mass index, ACS = acute coronary syndrome, PCI = percutaneous coronary intervention, CABG = coronary artery bypass grafting, CVA = cerebrovascular accident, PAD = peripheral arterial disease, EF = ejection fraction, ACE = angiotensin-converting enzyme, UAP = unstable angina pectoris, FU = follow-up, CAD = coronary artery disease, hsTnI = high-sensitivity troponin I, NTpro-BNP – N-terminal pro-brain natriuretic peptide.

Table 2. Characteristics of hematological parameters included in the prognostic set.

	Value Alive	Value Deceased	p-value difference	HR (95% CI)	p-value
Leukocyte count (10^9 cells/L)	2.32 [1.91, 2.86]	2.42 [1.94, 3.11]	0.224	1.25 (1.12-1.39)	<0.001
MCHCr (mmol/L)	15.73 [15.28, 16.22]	15.24 [14.83, 15.68]	<0.001	0.65 (0.50-0.86)	0.003
RBC green (FL1) fluorescence (AU)	17.52 [17.01, 18.03]	17.76 [17.30, 18.12]	0.011	1.51 (1.15-1.97)	0.003
% neutrophils (%)	5.97 [5.32, 6.60]	6.29 [5.74, 7.00]	0.001	1.37 (1.07-1.75)	0.012
% large* RBCs (%)	0.71 [0.46, 1.08]	1.03 [0.71, 2.00]	<0.001	1.17 (1.03-1.34)	0.019
% monocytes (%)	3.18 [2.65, 3.81]	3.58 [2.58, 4.19]	0.075	1.28 (1.04-1.59)	0.023
Neutrophil complexity CV (%)	7.27 [6.70, 7.89]	7.31 [6.65, 8.31]	0.377	1.31 (1.03-1.67)	0.026

Medians and interquartile ranges of hematological parameters are shown for alive and deceased patients. The multivariable adjusted hazard ratios for all-cause mortality are shown for each 1-SD increase the hematological parameter and derived from a model containing: age, sex, diabetes, hypercholesterolemia, smoking status, indication for angiography, angiographic CAD severity, history of PCI, history of ACS, kidney failure, treatment following angiography and the other hematological parameters shown in the table. The p-values in the far right column correspond to significance of the multivariable adjusted hazard ratios in the prior column.

Abbreviations: MCHCr = reticulocyte mean corpuscular hemoglobin concentration, RBC = red blood cell, AU = arbitrary units, CV = coefficient of variation. *>120 fL.

Table 3. Measures of improvement of all-cause mortality prediction.

In addition to clinical characteristics	IDI	p-value IDI	cNRI	p-value cNRI
Hematology	0.07 (0.03-0.14)	<0.001	0.37 (0.19-0.49)	<0.001
hsTnI	0.00 (0.00-0.00)	0.817	-0.07 (-0.16-0.20)	0.970
NTpro-BNP	0.02 (0.00-0.06)	0.040	0.03 (-0.14-0.22)	0.625
hsTnI + NTpro-BNP	0.02 (0.00-0.07)	0.066	0.02 (-0.13-0.20)	0.671
Hematology + hsTnI + NTpro-BNP	0.09 (0.05-0.17)	<0.001	0.44 (0.24-0.53)	0.007
In addition to clinical and hematological parameters				
hsTnI	0.01 (0.00-0.02)	0.113	0.16 (-0.18-0.31)	0.292
NTpro-BNP	0.01 (-0.01-0.06)	0.186	-0.08 (-0.25-0.14)	0.777
hsTnI + NTpro-BNP	0.02 (0.00-0.06)	0.093	-0.03 (-0.23-0.27)	1

Abbreviations: IDI = integrated discrimination improvement, cNRI = continuous net reclassification improvement, hsTnI = high-sensitivity troponin I, NTpro-BNP – N-terminal pro-brain natriuretic peptide.

Supplemental table 1. Parameters available and selected in the Utrecht Patient Oriented Database (UPOD).

UPOD parameters	Not intercorrelated (selected for further analysis)
Leukocyte count	X
Neutrophil count	
Lymphocyte count	
Monocyte count	X
Eosinophil count	
Basophil count	
Neutrophil %	X
Lymphocyte %	X
Monocyte %	X
Eosinophil %	X
Basophil %	X
Red blood cell count	
Hemoglobin concentration	X
Mean corpuscular volume	
Red blood cell distribution width	X
Mean corpuscular hemoglobin	X
Mean corpuscular hemoglobin concentration	
Hematocrit	
Platelet count	
Mean platelet volume	X
Plateletcrit	X
Platelet distribution width	X
Reticulocyte count	X
Reticulocyte %	
Immature reticulocyte fraction	
Mean neutrophil cell size	X
Mean neutrophil complexity	X
Mean neutrophil lobularity	X
Mean neutrophil granularity	X
Mean neutrophil red fluorescence	
CV of neutrophil cell size	X
CV of neutrophil complexity	X
CV of neutrophil lobularity	X
CV of neutrophil granularity	X
CV of neutrophil red fluorescence	X
Mean lymphocyte cell size	X
Mean lymphocyte complexity	X
CV of lymphocyte cell size	X
CV of lymphocyte complexity	X
Mean platelet complexity	
Mean platelet granularity	
CV of platelet complexity	
CV of platelet granularity	X
Mean red blood cell complexity	
CV of red blood cell complexity	
Mean red blood cell green (FL1) fluorescence	X
CV of red blood cell green (FL1) fluorescence	X
Reticulocyte mean corpuscular hemoglobin concentration	
Hemoglobin distribution width	X
Reticulocyte mean corpuscular hemoglobin	X
Reticulocyte mean corpuscular volume	
% red blood cells with hemoglobin concentration <28g/dL	
% red blood cells with hemoglobin concentration >41g/dL	
% red blood cells with volume >120 fL	X
% red blood cells with volume <60 fL	
Reticulated platelet count	X

All available UPOD parameters (n=56) are listed. The ones marked with 'X' were not significantly intercorrelated with other parameters (n=34) in hierarchically clustered heatmap analysis and therefore selected for further analysis.

CV= coefficient of variation.