

Clustering of blood cell count abnormalities and future risk of death

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Abstract

Background: The identification of novel predictors of poor outcome may help stratify cardiovascular risk. Aim was to evaluate the individual contribution of blood cell count parameters, as well as their clustering, on the risk of death and cardiovascular events over the long term in the population-based Malmö Diet and Cancer Study cohort.

Methods: In 30,447 individuals (age 57 ± 8 years), we assessed the incidence of all-cause death (primary endpoint) and major adverse cardiovascular events (MACE, secondary outcome measure) according to absence or presence of one, two and three factors at baseline out of the following: anaemia, leukocytosis and thrombocytosis. Median follow-up was 16 years.

Results: The percentages of all-cause death were 19.5% in individuals without factors, 21.3% in those with one factor, 27.4% with two and 46.4% with three (log-rank test $P < .001$). The crude incidence of MACE was 28.0%, 29.2%, 35.5% and 57.1%, respectively (log-rank test $P < .001$). At multivariate analysis, we found a stepwise increase in overall mortality with increasing number of prevalent factors (one factor: HR 1.23, 95% CI 1.14–1.31, $P < .001$; two factors: 1.61, 1.37–1.89, $P < .001$; three factors: 2.69, 1.44–5.01, $P = .002$, vs no factor). Similar findings were observed for the incidence of MACE (one factor: adjusted HR 1.18, 95% CI 1.11–1.24, $P < .001$; two factors: 1.52, 1.33–1.76, $P < .001$; three factors: 2.03, 1.21–3.67, $P < .001$, vs no factor).

Conclusions: The easily assessable clustering of anaemia, leukocytosis and thrombocytosis heralds higher incidence of death and adverse cardiovascular events.

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KEY WORDS

anaemia, blood cell count, cardiovascular events, mortality, population-based study

1 | INTRODUCTION

Stratification of cardiovascular risk is crucial to improve effectiveness of preventive strategies tackling the global burden of cardiovascular disease. The identification of novel predictors of poor outcome may help stratify cardiovascular risk, both in unselected populations and in selected patients with prevalent cardiovascular diseases. This better stratification enables to put into action more complete and effective strategies of cardiovascular prevention.

Inflammation is involved in the atherosclerotic process, including plaque rupture; a hyper-inflammatory status is associated with an increased incidence of major adverse cardiovascular events (MACE) and treatments able to decrease inflammation improved cardiovascular outcome both in primary and secondary prevention.^{1–5} An elevated white cell count indicates a hyper-inflammatory status; it has been correlated with thrombotic events and a more extensive prevalent coronary artery disease.^{6,7} Moreover, leukocytosis has been also correlated with impaired blood rheology in healthy volunteers and elevated levels of coagulation factors in population-based studies.^{8,9} However, reactive changes in platelet and red cell count may also reflect the individual's inflammatory response.¹⁰ The interplay between chronic inflammation and thrombosis is a major determinant of athero-thrombotic events, and there is mounting evidence that platelets interact with leucocytes and may be the link between inflammation and thrombosis.^{11–13} Platelet reactivity is increased in subjects with elevated platelet cell count and thrombocytosis has been recently associated with higher mortality and risk of cardiovascular events.^{14–16} Finally, a *J-shaped* and *U-shaped* association between haemoglobin levels and mortality has been observed.^{17,18}

From a broader perspective, changes in the different parameters of the complete blood cell count can report on the individual's clinical status and predict subsequent cardiovascular events.¹⁹ To date, however, no investigation specifically evaluated the individual contribution and the clustering of anaemia, leukocytosis and thrombocytosis on the risk of future cardiovascular events and death during a long-term follow-up in a prospective, population-based study. Notably, these parameters from the blood cell count are easily and rapidly obtainable by a simple and inexpensive blood test. We explored the abovementioned issue in the large cohort of the Malmö Diet and Cancer Study.

2 | METHODS

2.1 | Study cohort

All women born between 1923 and 1950 and all men born between 1923 and 1945 from the city of Malmö, Sweden (total population: 330,000) were asked to participate the prospective Malmö Diet and Cancer Study. The recruitment rate was approximately 40%. A total of 30,447 subjects were enrolled and underwent a baseline evaluation between 1991 and 1996. Complete description of screening and inclusion procedures has been previously described.²⁰ Patients and the public were not involved in any way.

2.2 | Definitions of clinical features

All participants received a baseline measurement of body weight, height and blood pressure (BP). Individuals filled a questionnaire on lifestyle (including smoking status), past clinical history and medical treatments. Arterial hypertension was classified as a systolic BP ≥ 140 mm Hg and/or a diastolic BP ≥ 90 mm Hg or the utilization of antihypertensive agents. Diabetes mellitus was defined as a physician-reported diagnosis or as therapy with antidiabetic drugs. Prevalent heart failure, atrial fibrillation, cancer, stroke and myocardial infarction (MI) were classified as self-reported, physician's diagnosis or based upon case-retrieval from the Swedish National Patient Register, previously known as the Swedish National Hospital Discharge Register (SNHDR). The study complied with the Declaration of Helsinki and was approved by the regional Ethics Committee.

2.3 | Blood sampling and assessment of the outcome measures

Blood samples for the measurement of the blood cell count were obtained at the time of the baseline visit, between 7:00 and 9:00 AM, after overnight fasting. For the purpose of this analysis, the following parameters were considered as valid: anaemia, with haemoglobin value <12 g/dL if female or <13 g/dL if male; leukocytosis, with a white cell count $>10^3$ cells per μL ; thrombocytosis, with platelet count $>275 \times 10^9 \text{ L}^{-1}$. In particular, the entire cohort was stratified by absence of these factors (anaemia, leukocytosis and

thrombocytosis) and presence of one, two or three factors. The abovementioned cut-off values of haemoglobin and white cell count were based on the available literature^{21,22}; the cut-off of platelet count $>275 \times 10^9 \text{ L}^{-1}$ corresponds to the value that in a recent, prospective, cohort study from our group was associated with the highest risk of adverse cardiovascular events and mortality.¹⁶

Participants were followed through 31 December 2014 by linking a unique 10-digit personal identification number with the SNHDR, Swedish National Cause of Death Register (SNCDR) and Stroke Register of Malmö (STROMA). Diagnoses in the SNHDR are coded as primary or contributory and in the SNCDR as underlying or contributory cause of death, both using the International Code for Diseases (ICD). The 9th edition (ICD-9) was applied between 1987 and 1996 and the 10th edition (ICD-10) from 1997 until present. The cohort was followed from participation date until the time of a study endpoint, until the end of follow-up or emigration, whichever occurred first. Event-free individuals ($n = 116$; 0.4%) who emigrated from Sweden before 31 December 2014 had the date of emigration as the last follow-up date.

Primary endpoint was the incidence of all-cause death according to the number of blood cell count factors present at baseline. Secondary endpoint was the composite outcome measure including MACE (cardiovascular death, MI, ischaemic stroke) and its individual components. Cardiovascular mortality was defined as death resulting from acute MI, heart failure, stroke, pulmonary embolism or other cardiovascular causes. Fatal or nonfatal MI or death due to coronary heart disease were classified following ICD9-410 and ICD10-I21 codes in SNHDR, and codes 410, 412, and 414 (ICD9) or I21-I23 and I25 (ICD10) in SNCDR. Ischaemic stroke was defined with ICD9-434 and ICD10-I63 codes. STROMA was utilized for retrieval of stroke cases up to 2010. All strokes in STROMA were validated by review of hospital records. In addition, SNHDR and SNCDR were used to retrieve stroke cases after 2010 or who had moved away from Malmö. The validity of register-based diagnosis of ischaemic stroke and coronary events in SNHDR were found to be high.²³

2.4 | Statistical analysis

Characteristics of the study population at baseline are reported as mean \pm standard deviation for continuous variables and frequency (percentage) for categorical variables. Kolmogorov-Smirnov test was performed to check the normal distribution of continuous variables. Differences in baseline characteristics across different clustering of blood cell count parameters were evaluated by one-way analysis of variance or Pearson's chi-square test, as appropriate. Time-to-event analyses by Kaplan-Meier estimator and log-rank test were performed. The Schoenfeld residuals test was used to check

the proportional hazards assumption. Hazard ratios (HR) with 95% confidence intervals (CIs) were computed from Cox models to estimate the magnitude of the increased risk of adverse events in individuals with the presence of one, two and/or three blood cell count parameters, with the absence of any such factor as the reference. The selection of confounding variables for adjustment was based on the identification of risk factors for measured outcomes, further encompassing clinical meaningful variables due to biological plausibility to interact with the observed effect size estimates.²⁴ All multivariate models were therefore adjusted for the following potential confounders: age, sex, body mass index, diabetes mellitus, smoking status, prevalent atrial fibrillation, history of congestive heart failure, prevalent cancer, previous stroke, previous MI, antihypertensive drug therapy, lipid-lowering treatment, antidiabetic therapy and antiplatelet treatment. For subgroup analyses, all variables were dichotomized (yes/no) and appropriate interaction terms were added to the fully adjusted model to evaluate differences of effect. All calculations were performed by the SPSS 23 software, with P values $<.05$ (two-tailed) being considered significant.

3 | RESULTS

As mentioned above, the Malmö Diet and Cancer Study population was stratified according to the presence of the following factors at baseline: anaemia, leukocytosis or thrombocytosis. A total of 23,343 individuals had no factor, 6358 had one factor, 718 had two factors and 28 three factors. Across increasing number of factors (Table 1), age and diastolic blood pressure were progressively lower, whereas the prevalence of female sex, smoking, diabetes and prevalent MI, as well as body mass index and the proportion of subjects who were taking beta-blockers, diuretics and aspirin, were higher. Individuals were followed for a median time of 16.1 years (interquartile range 14.8-17.7), corresponding to 466,853 person-years. The absolute numbers of subjects with incident adverse events were as follows: 6113 individuals with all-cause death, 2045 with cardiovascular death, 2468 with MI and 2289 with ischaemic stroke.

The percentages of all-cause death were 19.5% in individuals without factors, 21.3% in those with one factor, 27.4% with two factors and 46.4% with three factors (log-rank test $P < .001$) (Table 2). Kaplan-Meier curves for overall mortality indicated in Figure 1. In particular, mean survival was 17.9 years (standard error 0.03) in individuals without factors and 14.6 years (standard error 1.13) in those with three factors.

Crude incidence of MACE was also higher with increasing number of factors (28.0%, 29.2%, 35.5% and 57.1%, respectively; log-rank test $P < .001$) (Table 2). Separate analyses for individual components of the composite outcome measure of

TABLE 1 Baseline characteristics according to the number of factors present at baseline

Variables	No factor present (N = 23 343)	One factor present (N = 6358)	Two factors present (N = 718)	Three factors present (N = 28)	P value
Age at recruitment (y)	57.8 ± 7.5	56.8 ± 7.9	55.0 ± 8.0	56.4 ± 9.2	<.001
Body mass index (kg/m ²)	25.9 ± 4.0	25.7 ± 4.1	25.4 ± 4.5	27.0 ± 4.7	.001
Female sex	13,257 (56.8)	4528 (71.2)	521 (72.6)	20 (71.4)	<.001
Systolic blood pressure (mm Hg)	140.1 ± 20.0	141.0 ± 20.3	141.7 ± 19.7	145.7 ± 20.7	.506
Diastolic blood pressure (mm Hg)	85.7 ± 10.0	85.3 ± 10.0	84.6 ± 10.0	84.4 ± 8.5	.002
Arterial hypertension	4055 (17.4)	1098 (17.3)	119 (16.6)	7 (25.0)	.687
Current smoking	5727 (26.1)	1999 (33.9)	348 (51.7)	13 (54.2)	<.001
Diabetes mellitus	683 (3.5)	181 (3.4)	32 (5.2)	1 (3.6)	.129
Prevalent myocardial infarction	493 (2.1)	93 (1.5)	11 (1.5)	3 (10.7)	<.001
Prevalent heart failure	58 (0.3)	25 (0.4)	4 (0.6)	—	.131
Prevalent stroke	247 (1.1)	74 (1.2)	11 (1.5)	1 (3.6)	.421
Prevalent atrial fibrillation	251 (1.1)	53 (0.8)	8 (1.1)	—	.358
Prevalent cancer	1415 (6.1)	417 (6.6)	61 (8.5)	1 (3.6)	.030
Drugs					
ACE inhibitors	645 (2.8)	178 (2.8)	16 (2.2)	1 (3.6)	.830
Beta-blockers	2239 (9.6)	569 (9.0)	46 (6.4)	6 (20.7)	.002
Diuretics	1266 (5.4)	408 (6.4)	55 (7.3)	—	.001
Calcium-antagonists	1072 (4.6)	291 (4.6)	38 (5.3)	4 (14.3)	.080
Lipid-lowering	672 (2.9)	192 (3.0)	18 (2.5)	2 (7.1)	.468
Antidiabetic	385 (1.6)	95 (1.5)	14 (1.9)	—	.636
Ticlopidine	12 (0.1)	3 (0.1)	—	—	.944
Aspirin	547 (2.5)	113 (1.9)	24 (3.6)	2 (7.1)	.002
Dipyridamole	39 (0.2)	6 (0.6)	2 (0.3)	—	.492

Note: Values are expressed as mean ± standard deviation or n (%). Significant P values are in bold.

TABLE 2 Crude incidence of adverse events during follow-up according to the number of factors present at baseline

	No factor present (N = 23,343)	One factor present (N = 6358)	Two factors present (N = 718)	Three factors present (N = 28)	Log-rank P
All-cause death	4547 (19.5)	1356 (21.3)	197 (27.4)	13 (46.4)	<.001
MACE	6538 (28.0)	1854 (29.2)	255 (35.5)	16 (57.1)	<.001
Cardiovascular death	1550 (6.6)	435 (6.8)	55 (7.7)	5 (17.9)	.005
MI	1910 (8.2)	492 (7.7)	61 (8.5)	5 (17.9)	.070
Stroke	1749 (7.5)	478 (7.5)	57 (7.9)	5 (17.9)	.042

Note: Values are expressed as n (%). Significant P values are in bold.

Abbreviations: MACE, Major adverse cardiovascular events; MI, Myocardial infarction.

MACE yielded consistent results (Table 2). Kaplan-Meier curves for MACE are depicted in Figure 2: MACE-free survival was 16.8 years (standard error 0.03) in individuals without factors and 13.3 years (standard 1.13) in those with three factors.

With the absence of factors as reference, multivariate analysis demonstrated a stepwise increase in the risk of overall death by increasing number of factors (one factor: HR 1.23, 95% CI 1.14-1.31, $P < .001$; two factors: 1.61, 1.37-1.89, $P < .001$; three factors: 2.69, 1.44-5.01, $P = .002$; Figure

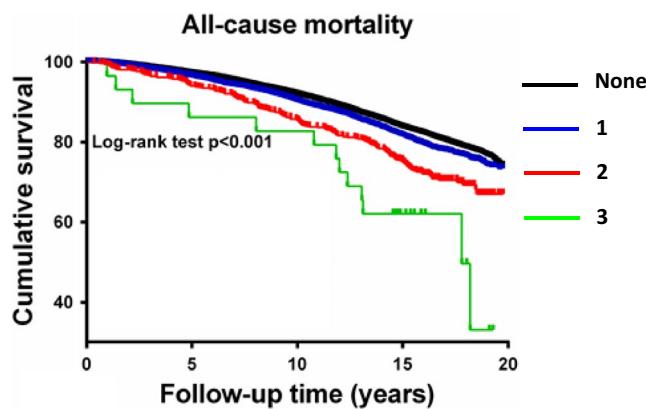


FIGURE 1 Survival curves for all-cause mortality according to the number of factors present at baseline (none; 1; 2; 3)

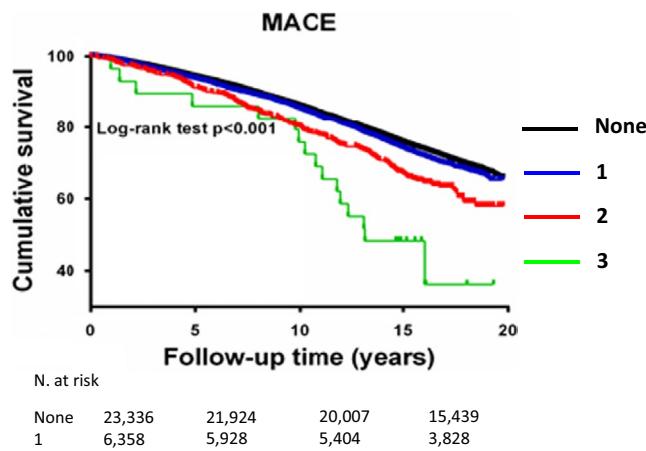


FIGURE 2 Survival curves for MACE according to the number of factors present at baseline (none; 1; 2; 3). MACE, Major adverse cardiovascular events

S1). In patients with two or three factors present, the adjusted HR for all-cause mortality vs no factor was 1.75, 1.49-2.05 ($P < .001$; Figure 3).

Among individuals with two factors, the highest incidence of all-cause death was predicted by the association of anaemia plus leukocytosis: adjusted HR 2.45, 1.63-3.93, $P < .001$. Adjusted HR for overall mortality in subjects with anaemia plus thrombocytosis was 2.34 (1.61-3.42, $P = .009$), and in those with elevation of platelet count and white cell count was 1.94 (1.36-2.52, $P < .001$).

As a natural decrease in the number of platelets along with the increasing age occurs, we have also performed a sensitivity analysis for the primary endpoint of all-cause death by using an age-dependent threshold of platelet count, instead of a fixed threshold, for defining an alteration in platelet count

(Table S1).²⁵ Here the adjusted Hazard ratios for all-cause death by using the 75th percentile of platelet count as platelet threshold across different tertiles of age were consistent with the primary analysis.

A significant relationship with number of factors present was also demonstrated for MACE occurrence (one factor: adjusted HR 1.18, 1.11-1.24, $P < .001$; two factors: 1.52, 1.33-1.76, $P < .001$; three factors: 2.03, 1.21-3.67, $P < .001$, vs no factor) (Figure S1). In patients with two or three factors present, the adjusted HR for MACE vs no factor was 1.54, 1.34-1.77 ($P < .001$) (Figure 3). Consistent results were found for the individual components of the MACE outcome measure (Figure 3).

At multivariate analysis, the increase in mortality of subjects with two or three parameters compared to those without any parameter tended to be more pronounced in the subgroup with prevalent MI (HR 2.72, 1.29-5.73, $P = .008$) vs no prevalent MI (HR 1.71, 1.45-2.01, $P < .001$), but P for interaction was not significant (.59) (Figure 4). The poorer outcome in individuals with two or three factors was regardless of prevalent diabetes. Interestingly, there was a quantitative interaction between increased risk of all-cause death in individuals having two or three factors and smoking status (current smoking: HR 1.46, 1.17-1.82; no current smoking: HR 2.16, 1.73-2.71; P for interaction .011).

4 | DISCUSSION

In this large, prospective, cohort study, we documented that, in community-dwelling middle-aged individuals, the clustering of anaemia, leukocytosis and thrombocytosis is strongly associated with a higher incidence of mortality and adverse cardiovascular events.

Variations in parameters of the blood cell count can reflect the individual's clinical status and predict subsequent cardiovascular events. An analysis from the population-based EPIC-NL cohort reported the individual contribution of different parameters expressed in the blood cell count on negative outcome²⁶; in this study, the strongest association with poorer outcome was observed in individuals having an elevated white cell count at baseline. Furthermore, the combined use of a risk stratification model based on both blood cell count and clinical scores in patients with acute coronary syndromes yielded a more accurate prediction of adverse events.²⁷ To date, no investigation has specifically explored the contribution of clustering of parameters expressed in the blood cell count on the risk of cardiovascular events and death during long-term follow-up in a prospective, population-based study.

We investigated the predictive role of clustering of anaemia, leukocytosis and thrombocytosis at baseline on outcome in the large cohort of the Malmö Diet and Cancer Study. We found a

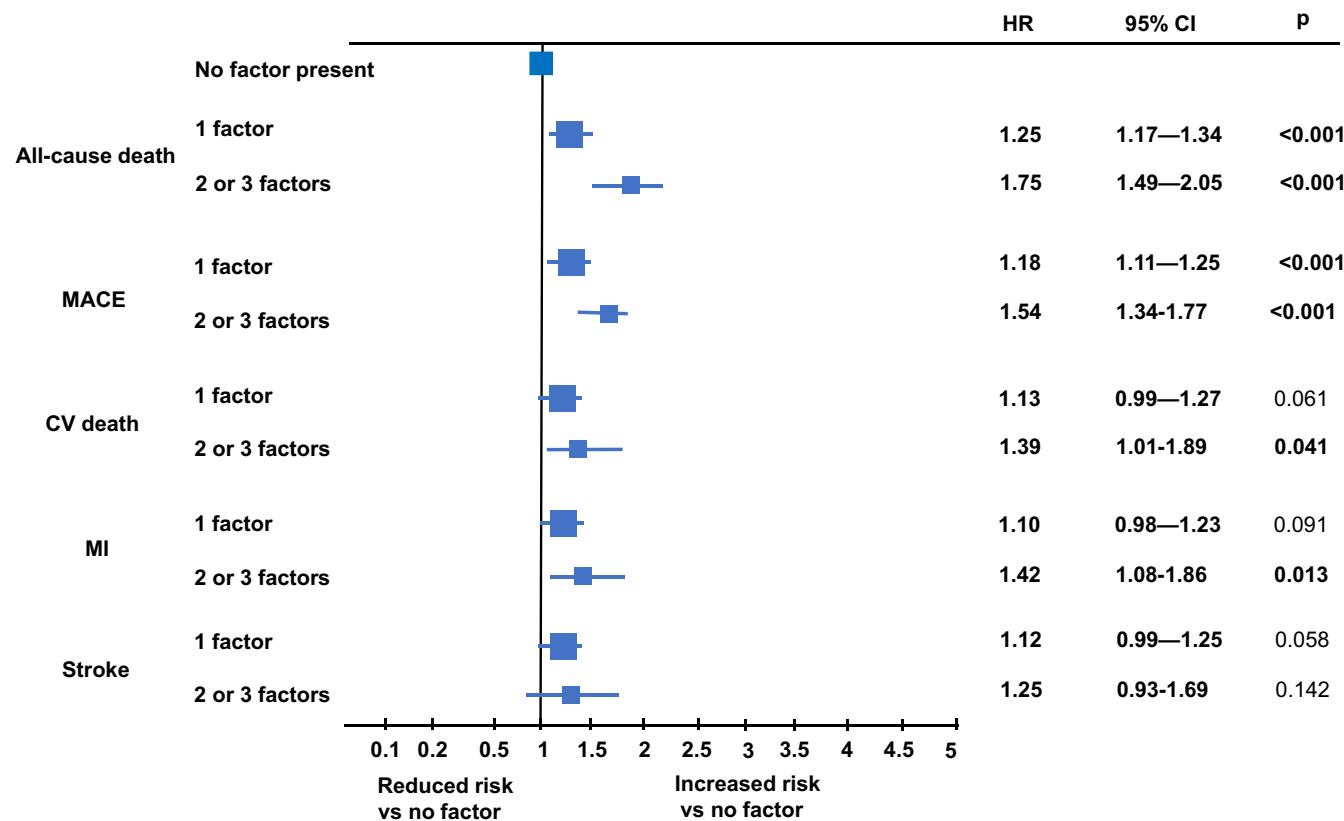


FIGURE 3 Adjusted hazard ratios (HR) for the various outcome measures by number of factors present (1 factor and 2 or 3 factors vs no factor). CV, Cardiovascular; MACE, Major adverse cardiovascular events; MI, Myocardial infarction

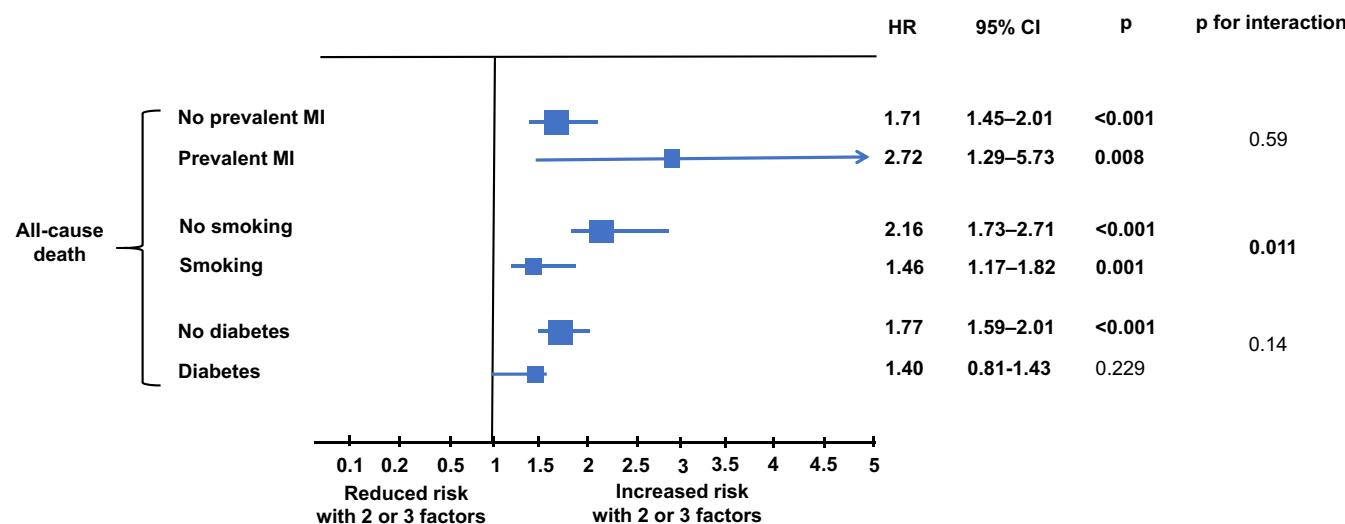


FIGURE 4 Adjusted hazard ratios (HR) for all-cause death by numbers of factors present (2 or 3 factors vs no factor) in subgroups

stepwise increase in the risk of both all-cause death and MACE by increasing number of parameters present. Notably, mean survival in individuals with all three factors present at baseline was 3.3 years lower compared to those without factors. Using the absence of blood cell analysis-derived risk factors as reference, multivariate analysis revealed a 54% relative increase in the risk of MACE and a 75% increase in all-cause mortality among individuals having two or three parameters present. The

association between number of factors and reduced survival was independent of prevalent MI at baseline, but was stronger in individuals with previous history of MI and in those without current smoking. Importantly, in the individual setting an accurate cardiovascular risk stratification based on conventional risk factors can be difficult, especially in subjects without ‘stigmata’ of very low- or very high-risk profile, in whom an integrative evaluation might provide an additive value. This is a

valuable approach inasmuch as it utilizes co-parameters easily obtainable, as demonstrated in our study, where an information derived from a simple, rapid and inexpensive blood test predicted outcome independently of conventional cardiovascular risk factors.

According to our data, anaemia, leukocytosis and thrombocytosis are a proxy of impaired health status; in particular, they can reflect a hyper-inflammatory status. Other mechanisms specifically linking leukocytosis and coronary events might be leucocyte-exacerbated coronary thrombus formation, leucocyte-mediated microvascular injury and release of vascular toxic factors.²⁸ Thrombocytosis is often secondary to anaemia, cancer, infective or systemic inflammatory diseases; therefore, a high platelet count has also to be considered as a subclinical marker of possible serious concealed conditions. However, platelets could be directly involved in amplifying endothelial dysfunction/damage and promoting angiogenesis and fibrosis²⁹; thus, increased platelet concentrations, even below pathologically abnormal values, might contribute to the initiation, progression and complication of atherosclerosis. The presence of anaemia frequently reflects older age and pre-existing conditions, such as renal failure, bone marrow diseases, cancer or chronic systemic disorders; notably, previous data showed that pro-inflammatory cytokines interact with erythropoietin in the bone marrow, causing a lower production of red blood cells.³⁰ Therefore, anaemia represents a marker of frailty and has been related to increased morbidity and mortality in various clinical settings, including patients with coronary artery disease.^{17,18} Interestingly, in our investigation the adjusted risk of overall mortality among subjects with two factors present was lower for the association of thrombocytosis plus leukocytosis, higher for anaemia plus thrombocytosis and highest for anaemia plus leukocytosis. Indeed, mean erythrocyte lifespan is long and haemoglobin values are relatively stable overtime; thus, low haemoglobin values are a reliable and more stable marker of frailty and/or high inflammatory status. However, specific anaemia-related conditions could also increase the risk of ischaemic events, that is, decreased oxygen supply to the myocardium downstream of pre-existing coronary stenoses, increased myocardial oxygen demand due to a greater cardiac output to maintain adequate blood oxygen levels, rheologic modifications leading to thrombosis and the need for transfusions, with attendant pro-inflammatory milieu and immunologic effects.

Our study has strengths and limitations. Strengths are as follows: the robustness of the data obtained from a large, real-life, adult general population, where severely ill individuals were comprised; the long follow-up duration, with large number of person-years; the reliability of prospectively collected data, with limited loss to follow-up; the stability of the health care and reporting system over time, where adverse events during follow-up were carefully counted

through medical history and hospitalization records.^{31,32} Limitations are the risk of inclusion bias and residual confounding, and the lack of adjustment for all potential confounders. Approximately 70% of all stroke cases were validated by review of hospital records, but no individual event adjudication was performed for MI. However, many previous studies have shown that the validity of MI in SNHDR is high and fully acceptable for epidemiological studies.²³ Furthermore, in the majority of cases no specific information on conditions potentially causing variations in blood cell count was available. The assessment of the blood cell count was done by a single measurement; thus, overtime changes of haemoglobin, white cell count and platelet count, especially occurring in presence of multiple co-morbidities and during a long follow-up period, were not captured. We cannot exclude that a proportion of participants after the enrolment moved across the defined thresholds of blood parameters. However, age-related changes should proportionally affect all participants regardless of baseline status. Incident cardiovascular diseases did not modify the risk mediated by abnormal blood parameters, as these individuals had already had the endpoint of interest and were censored at that time. The cut-off values chosen might be questionable, but they reflect currently available evidence on the association of haemoglobin levels, white cell count and platelet count with cardiovascular events. The associations observed in this investigation do not prove causation and a causal effect of clustering of blood cell count components on mortality and morbidity cannot be specifically evaluated in an observational study. However, the linearity of the event curves according to the number of factors present and the adjustment for potential confounders support the hypothesis that the clustering of anaemia, leukocytosis and thrombocytosis might represent pathophysiological mechanisms acting over the long time beside acting as proxies for other unmeasured disorders. Furthermore, the number of patients with three impaired factors was limited, and therefore, the results regarding this specific subgroup, albeit statistically significant, should be taken with caution. Finally, we were not able to present the burden of co-morbidities of our population for example by the age-adjusted Charlson comorbidity index, as such index requires baseline information that was not available in our dataset.

In conclusion, our study indicates that the clustering of anaemia, leukocytosis and thrombocytosis is independently associated with reduced survival, regardless of the history of previous MI, and increased risk of cardiovascular events. Thus, blood cell count may represent an additive reliable, nonexpensive and readily available tool for stratification of the cardiovascular risk. Our findings support a practical, comprehensive, multiparametric evaluation of the cardiovascular risk aimed to individually tailored approaches of primary and secondary cardiovascular prevention.

CONFLICTS OF INTEREST

GP: speaker/consultant/advisory board for Amgen, Sanofi, Bayer, Boehringer-Ingelheim, BMS-Pfizer, Daiichi Sankyo, Astra Zeneca, Sigma-Tau, Malesci, PIAM and MSD. VL: no disclosure. GDM: no disclosure. FR: no disclosure. GR: speaker/consultant/advisory board for Bayer, Boehringer Ingelheim, BMS/Pfizer and Daiichi-Sankyo. OM: no disclosure. GE: no disclosure. VH: Educational funding from Boston Scientific. Shareholder in AstraZeneca and Swedish Orphan Biovitrum. RDC: fees, honoraria and research funding from Sanofi-Aventis, Boehringer Ingelheim, Bayer, BMS/Pfizer, Daiichi-Sankyo, Novartis, MSD and Portola. AF: Speaker/consultant/advisory board for Medtronic. Honoraria and royalties from Cardiome and ThermoFisher.

AUTHOR'S CONTRIBUTIONS

G. Patti designed the study; F. Ricci contributed to data collection; the analysis was done by G. Di Martino; interpretation of the results was done by G. Patti, V. Lio, F. Ricci, G. Renda, O. Melander, G. Engström, V. Hamrefors, R. De Caterina and A. Fedorowski; the paper was drafted by G. Patti; critical revision of the paper for important intellectual content was done by all authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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