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Original Paper

Low CD34$^+$ cells, high neutrophils and the metabolic syndrome are associated with an increased risk of venous thromboembolism

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Abstract

The relationship between MetS (metabolic syndrome), levels of circulating progenitor/immune cells and the risk of VTE (venous thromboembolism) has not yet been investigated. We studied 240 patients with previous VTE and 240 controls. The presence of MetS was identified according to NCEP ATP III guidelines and flow cytometry was used to quantify circulating CD34$^+$ cells. VTE patients showed higher BMI (body mass index), waist circumference, triacylglycerol (triglyceride) levels, blood glucose, hs-CRP (high-sensitivity C-reactive protein) and lower HDL-C (high-density lipoprotein cholesterol) levels. The prevalence of MetS was significantly higher in VTE (38.3%) than in control individuals (21.3%) with an adjusted OR (odds ratio) for VTE of 1.96 ($P = 0.002$). VTE patients had higher circulating neutrophils ($P < 0.0001$), while the CD34$^+$ cell count was significantly lower among patients with unprovoked VTE compared with both provoked VTE ($P = 0.004$) and controls ($P = 0.003$). Subjects were also grouped according to the presence/absence of MetS (MetS$^+$ or MetS$^-$) and the level (high/low) of both CD34$^+$ cells and neutrophils. Very high adjusted ORs for VTE were observed among neutrophils_high/MetS$^+$ (OR, 3.58; $P < 0.0001$) and CD34$^+$_low/MetS$^+$ (OR, 3.98; $P < 0.0001$) subjects as compared with the neutrophils_low/MetS$^-$ and CD34$^+$_high/MetS$^-$ groups respectively. In conclusion, low CD34$^+$ blood cell count and high circulating neutrophils interplay with MetS in raising the risk for venous thromboembolic events.

Key words: CD34$^+$ cell, inflammation, leucocyte, metabolic syndrome, neutrophil, venous thromboembolism

INTRODUCTION

Prevalence of atherosclerosis and the risk of ischaemic cardiovascular events are increased among subjects with history of VTE (venous thromboembolism) [1–3]. Although mechanisms underlying this association have not yet been elucidated, a common substrate of endothelial dysfunction, chronic inflammation and pro-thrombotic state has been postulated [4]. In the last decade, several population studies clearly demonstrated a higher risk of cardiovascular mortality and morbidity among subjects with MetS (metabolic syndrome) [5], a clinical condition characterized by the clustering of several inter-related metabolic abnormalities. Of interest, features of MetS, such as low HDL-C (high-density lipoprotein cholesterol) levels, central obesity, hypertriglyceridaemia, increased BP (blood pressure) and mild hyperglycaemia have been shown to be more prevalent among subjects with a history of VTE [6–11]. However, factors driving the association between MetS and the risk of VTE are currently unknown.

The role played by bone-marrow-derived progenitor cells in vascular disease progression is a matter of intense investigation [12–14]. Clinical and basic science findings demonstrated that

Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; CRP, C-reactive protein; CT, computed tomography; DBP, diastolic BP; DVT, deep venous thrombosis; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity CRP; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; OR, odds ratio; PE, pulmonary embolism; SBP, systolic BP; SI, synergy index; TAG, triacyglycerol(s); VKA, vitamin K antagonist; VTE, venous thromboembolism; WBC, white blood cell.

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reduced blood levels of endothelial progenitor cells is accompanied by defective mechanisms of vascular repair and increased risk of arterial thrombotic events [15,16]. Moreover, it has been clearly shown that metabolic disorders, such as MetS and diabetes, are significantly associated with low levels of circulating progenitor cells, a reduction that might contribute in generating high risk atherothrombotic events observed in these clinical categories [17]. However, the role played by these cells in the pathogenesis of VTE has not so far been investigated.

MetS, and in particular abdominal obesity, has also been associated with the presence of subclinical chronic inflammation, a condition characterized by augmented circulating levels of inflammatory cytokines, such as CRP (C-reactive protein) and WBCs (white blood cells), especially neutrophils and monocytes [18,19]. In this context, a central role seems to be played by the adipose tissue, which releases a series of pro-inflammatory and pro-atherogenic mediators, potentially involved in insulin resistance, endothelial dysfunction and hypercoagulability [10,20]. However, no clinical data are available about a possible connection between metabolic disorders, levels of circulating WBCs and the risk of VTE.

For these reasons, we designed a case–control study that specifically investigated the potential contribution of MetS, levels of $CD34^+$ cells in the blood and circulating leucocytes in determining VTE risk.

MATERIALS AND METHODS

Population

We enrolled 240 patients who were referred to the Thrombosis Unit of the Cà Foncello Hospital in Treviso between October 2010 and April 2012 and had at least one objectively diagnosed episode of DVT (deep venous thrombosis) and/or PE (pulmonary embolism). DVT diagnosis was obtained by performing a complete lower limb CUS (compression ultrasonography) and was based on the observation of incomplete compressibility of the vein, including the distal districts. Among patients with clinical suspicion of PE the diagnosis was confirmed by ventilation-perfusion scintigraphy (V/Q scan) and/or CT (computed tomography) angiography. V/Q scans were considered diagnostic in the case of mismatched segmental perfusion defects, suggestive for high-probability PE. CT angiography was considered diagnostic for PE in the case of perfusion defects in the pulmonary vasculature at the segmental or proximal level. At the time of VTE diagnosis, all of the patients were hospitalized and treated with either intravenous adjusted dose of unfractionated heparin or fixed dose LMWH [low-molecular-mass ('weight') heparin]. All of the patients were then shifted to VKAs (vitamin K antagonists) for at least 3 months. The decision to prolong the VKA was made on an individual basis considering the risk of recurrent VTE. We considered as 'provoked VTE' the events associated with surgery, trauma, bone fracture, immobilization, acute medical disease, oral contraceptives, hormonal replacement therapy or pregnancy. We excluded history of cancer from the present analysis of patients. All of the VTE events without triggering condition were denoted as 'unprovoked'. We did not include in the study patients with VTE less than 3 months apart from the enrolment visit. Information about VTE characteristics were obtained from the medical record of the patients. In particular, we collected information about the type of VTE (unprovoked $n = 161$ or provoked $n = 79$), the site of DVT (proximal $n = 161$ or distal $n = 50$), the presence of PE ($n = 63$), history of recurrence ($n = 52$) and the presence of thrombophilia ($n = 86$). The screening for thrombophilic conditions was available for 181 patients and included: anti-thrombin III ($n = 5$), Factor V Leiden mutation ($n = 26$), prothrombin G20210A mutation ($n = 27$), protein C ($n = 9$) and protein S ($n = 14$) deficiency, and lupus anticoagulant ($n = 16$). Eleven patients displayed more than one defect. The average temporal distance between the VTE event and the enrolment in the study was 68.3 (range 4–156) months. An identical number of control subjects unrelated to patients were selected from healthy volunteers, health care personnel of the Hospital, blood donors and referred patients without evidence/history of VTE. Controls were selected to match as close as possible with cases for age and gender. VTE was not objectively excluded in the control group, although none of the subjects enrolled showed clinical signs of DVT or PE. As a partial confirmation of VTE absence among controls, all of the subjects did not report novel clinical events when they were called back to receive the results of the biochemical analyses. We excluded subjects with clinical and biochemical suspicion of local and/or systemic inflammatory disease from the study [in particular, we did not include subjects with hs-CRP (high-sensitivity CRP)>1 mg/dl and WBCs above the upper normal limit]. The study was carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association and was approved by the local Ethics Committee. All of the subjects enrolled gave written informed consent.

Data collection and biochemical analysis

Data collected from patients and controls included: age, gender, height, weight, waist circumference, BMI (body mass index), history of atherosclerotic cerebro-cardiovascular events (ischaemic stroke, transient ischaemic stroke and acute coronary syndrome), smoking status, known arterial hypertension, known diabetes, current use of antihypertensive drugs, oral anticoagulants, antiplatelet drugs and lipid-lowering treatment. Waist circumference was measured by using a measuring tape as described previously [21]. BP was measured in a sitting position and after 5 min of rest using a mercury sphygmomanometer of adequate cuff size. The mean of three measures 1 min apart was considered. Hypertension was defined as SBP (systolic BP) $\geqslant$140 mmHg, DBP (diastolic BP) $\geqslant$90 mmHg or the use of antihypertensive medication. Diabetes was defined as self-reported, the use of diabetes medication or fasting blood glucose $\geqslant$126 mg/dl. All VTE cases and controls underwent fasting blood sampling to measure: total cholesterol, HDL-C, TAG [triacylglycerols (triglycerides), glycaemia and creatinine. LDL-C (low-density lipoprotein cholesterol) levels were determined by using the Friedwald formula. eGFR (estimated glomerular filtration rate) was calculated using the MDRD (Modification of Diet in Renal Disease) equation.

According to the NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) criteria, the presence of MetS was identified with the presence of three or more of the

following characteristics: (i) SBP ⩾130 mmHg or DBP ⩾85 mmHg or ongoing antihypertensive therapy, (ii) fasting blood glucose ⩾100 mg/dl or ongoing hypoglycaemic therapy, (iii) waist circumference ⩾102 cm in men and ⩾88 cm in women, (iv) TAG ⩾150 mg/dl or ongoing treatment for hypertriglyceridaemia and (v) HDL-C ⩽40 mg/dl in men and ⩽50 mg/dl in women.

hs-CRP circulating levels were measured in serum samples collected and stored at −70°C until analysis. hs-CRP was determined by using a chemiluminescent immunometric assay (IMMULITE; Siemens), following the manufacturer's instructions.

WBC count and flow cytometry analysis

Blood samples for WBC count and flow cytometry analysis were processed within 2 h. The analysis also included measurement of haemoglobin levels and platelets. For each patient a detailed description of the WBC types was obtained by automated analyser (ADVIA 2120 Hematology System; Siemens Healthcare Diagnostics), including the count of circulating neutrophils, lymphocytes, monocytes, basophils and eosinophils. The number of circulating cells was expressed as numbers/mm^3.

Peripheral blood progenitor cells were analysed for the surface expression of CD34+ by flow cytometry as described previously in detail (intraclass correlation coefficient 0.94 and mean-centred coefficient of variation 6.3%) [15]. Briefly, cells were labelled with phycoerythrin-conjugated anti-CD34 (Becton Dickinson), and gated in the mononucleated cell fraction. In all samples, 1×10^6 events were acquired, scored using a FACSCanto flow cytometer (Becton Dickinson), and analysed using the FACSDIVA software program (Becton Dickinson).

Statistical analysis

Data are expressed as means ± S.D. or as a percentage, where appropriate. Comparison between groups for continuous variables was performed by using Student's t test or ANOVA followed by a post-hoc least significant difference test for normally distributed parameters and the Mann–Whitney U test for variables without normal distribution. A χ^2 test was used for comparison of categorical variables. A binary logistic regression analysis was performed to estimate univariate and multivariate OR (odds ratio) and 95% CIs (confidence intervals) of MetS for VTE. A similar analysis was performed to estimate the OR of each component of MetS. Patients with CD34+ cell count and neutrophil levels equal or below the median of the study population were classified as CD34+_low and neutrophils_low respectively. Subjects with circulating levels above the median were classified as CD34+_high and neutrophils_high. Cases and controls were then grouped according to the presence/absence of MetS and high/low levels of CD34+ or neutrophils. Crude and adjusted ORs for VTE were then computed in a binary logistic regression analysis. The Rothman SI (synergy index) was calculated to estimate the synergy effect for VTE between CD34+ cell count, neutrophil levels and MetS. All statistical analyses were performed using PAWS statistics 18.0 (SPSS).

RESULTS

Population characteristics

A total of 240 subjects with a history of VTE and 240 controls were studied. Demographic and laboratory characteristics of the population are summarized in Table 1. Individuals with previous objectively documented VTE and control subjects showed similar SBP and DBP levels, renal function, prevalence of active/previous smoking and history of atherosclerosis (Table 1). Compared with controls, patients with VTE showed significantly higher BMI, waist circumference, TAG levels and blood glucose. Total cholesterol and LDL-C were not significantly different between cases and controls, whereas HDL-C levels were reduced in VTE patients compared with controls ($P=0.017$). The frequency of diabetes was higher in VTE ($P=0.003$), although the overall prevalence in cases (9.6%) and controls (2.9%) was modest. As expected, a significant difference was observed in the number of subjects undergoing VKA treatment, which was almost exclusively found in the VTE group (48.7% compared with 0.8%). The use of anti-platelet agents was similar in the two groups ($P=0.25$), whereas the frequency of patients using lipid-lowering drugs was slightly higher in VTE patients (15.8% compared with 9.6%, $P=0.04$). A significant increase in hs-CRP levels was observed in cases compared with controls ($P=0.012$). All values are shown in Table 1.

MetS and VTE risk

The presence of MetS was identified in 92 (38.3%) of the 240 VTE cases and in 51 (21.3%) of the control individuals ($P<0.0001$) (see Table 2). The association between MetS and the risk of VTE remained significant even after adjustment for age, gender and hs-CRP levels ($P=0.002$), with an adjusted OR for VTE of 1.96 (95% CI, 1.28–3.00). In Table 2, we also reported the interaction between each component of MetS and the risk of VTE. In particular, the stronger levels of association were observed for elevated waist circumference [OR, 2.06 (95% CI 1.43–2.96); $P<0.0001$], fasting glucose ⩾100 mg/dl [OR, 2.00 (95% CI 1.35–2.95)] and TAG ⩾150 mg/dl [OR, 1.93 (95% CI 1.19–3.11)], findings that remain significant even after adjustment for age, gender and hs-CRP (Table 2).

Association of neutrophils and CD34+ cell count with VTE

Analysis of the blood cell constituents showed a significant increase in the number of circulating WBCs in VTE compared with controls ($P<0.0001$) (Table 1). The differential WBC count then revealed that only neutrophils ($P<0.0001$) and monocytes ($P=0.030$) were significantly increased in VTE subjects. No differences between cases and controls were seen for lymphocytes, eosinophils and basophils (Table 1). The differences in neutrophil levels between cases and controls remained significant even after adjustment for the ongoing medical treatment (use of aspirin, statins and anti-hypertensive drugs) and a smoking habit ($P<0.001$). In a logistic regression analysis including age, gender, the presence of MetS, hs-CRP, neutrophils, monocytes and smoking habit, only MetS ($P=0.015$), neutrophils

Table 1 Population characteristics
Values are means (S.D.), medians (interquartile range) or numbers (percentage).

Parameter	VTE	Controls	*P* value
n	240	240	
Age (years)	57.1 (14.4)	55.2 (13)	0.18
Male gender (*n*)	124 (51.7%)	116 (48.3%)	0.46
BMI (kg/m^2)	27.6 (4.8)	25.3 (4.2)	**<0.0001**
Waist circumference (cm)	101.2 (12.4)	93.6 (12.3)	**<0.0001**
Active/previous smokers (*n*)	116 (48.3%)	124 (51.7%)	0.43
History of atherosclerotic events (*n*)	12 (5%)	6 (2.5%)	0.14
SBP (mmHg)	134 (19)	132 (19)	0.24
DBP (mmHg)	88.0 (11.3)	86.6 (9.5)	0.13
eGFR male (ml/min per 1.73 m^2)	83.2 (23.1)	85.2 (17)	0.44
eGFR female (ml/min per 1.73 m^2)	82.5 (19.8)	84.5 (15.7)	0.39
Total cholesterol (mg/dl)	209.2 (40.9)	216.3 (40.5)	0.057
HDL-C (mg/dl)	58.4 (15.6)	62.1 (17.5)	**0.017**
LDL-C (mg/dl)	127.1 (38.1)	133.7 (36.7)	0.056
TAG (mg/dl)	101.0 (71.0–145.25)	86.0 (66.0–121.0)	**0.004**
Glycaemia (mg/dl)	95.0 (88.0–105.0)	93.0 (87.0–99.0)	**0.020**
Diabetes (*n*)	23 (9.6%)	7 (2.9%)	**0.003**
Arterial hypertension (*n*)	146 (60.8%)	100 (41.7%)	**<0.0001**
Therapy with statins (*n*)	38 (15.8%)	23 (9.6%)	**0.04**
Antiplatelet therapy (*n*)	24 (10%)	17 (7.1%)	0.25
Oral anticoagulants (*n*)	117 (48.7%)	2 (0.8%)	**<0.0001**
hs-CRP (mg/dl)	0.24 (0.09–0.50)	0.14 (0.06–0.34)	**0.012**
Platelets (*n*/mm^3)	257 379.1 (65667.3)	256 025.2 (72428.6)	0.83
Haemoglobin (mg/dl)	14.2 (1.4)	14.3 (1.2)	0.45
Leucocytes (*n*/mm^3)	6631.2 (1765.3)	5923.6 (1327.6)	**<0.0001**
Neutrophils (*n*/mm^3)	3798.9 (1435.4)	3174.8 (989)	**<0.0001**
Lymphocytes (*n*/mm^3)	2130.5 (634.5)	2077.5 (577.2)	0.34
Monocytes (*n*/mm^3)	454.73 (140.8)	427.6 (130.5)	**0.030**
Eosinophils (*n*/mm^3)	219.3 (196)	199.4 (130.2)	0.19
Basophils (*n*/mm^3)	44.9 (32.6)	46.7 (46.8)	0.62

Table 2 MetS and risk of VTE: frequency of each component and association with VTE

Parameter	VTE (*n* = 240)	Controls (*n* = 240)	OR (95% CI)	*P* value	Adjusted OR (95% CI)*	*P* value
MetS (*n*)	92 (38.3%)	51 (21.3%)	2.30 (1.53–3.45)	**<0.0001**	1.96 (1.28–3.00)	**0.002**
SBP $\geqslant$130 mmHg or DBP $\geqslant$85 mmHg (*n*)	193 (80.4%)	171 (71.3%)	1.65 (1.08–2.53)	**0.020**	1.57 (0.97–2.54)	0.066
HDL-C $\leqslant$40 mg/dl male or $\leqslant$50 mg/dl, female (*n*)	41 (17.1%)	30 (12.5%)	1.43 (0.86–2.39)	0.16	1.45 (0.87–2.43)	0.15
TAG $\geqslant$ 150 mg/dl (*n*)	55 (22.9%)	32 (13.3%)	1.93 (1.19–3.11)	**0.007**	1.92 (1.17–3.15)	**0.009**
Glycemia $\geqslant$100 mg/dl (*n*)	96 (40%)	60 (25%)	2.00 (1.35–2.95)	**<0.0001**	1.95 (1.29–2.93)	**0.001**
Waist circumferences $\geqslant$102 cm male or $\geqslant$88 cm female (*n*)	140 (58.3%)	97 (40.4%)	2.06 (1.43–2.96)	**<0.0001**	2.04 (1.91–2.95)	**<0.0001**

*Adjusted for age, gender and hs-CRP.

($P < 0.0001$) and hs-CRP ($P = 0.019$) were significantly associated with VTE risk. When the analysis was performed considering as covariates each component of MetS, only central obesity ($P = 0.024$), blood glucose ($P = 0.043$), hs-CRP ($P = 0.024$) and neutrophils ($P < 0.0001$) emerged as independent predictors of VTE (see Supplementary Table S1 at http://www.clinsci.org/cs/125/cs1250211add.htm). Analysis of neutrophil levels in different subgroups of VTE showed no significant difference on the basis of type of VTE (provoked compared with unprovoked), DVT extent (distal compared with proximal), the presence of PE, thrombophilia, history of recurrence and use of VKAs.

Table 3 ORs for VTE

Parameter	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)*	*P* value
CD34$^+$_high/MetS$^-$	Reference		Reference	
CD34$^+$_low/MetS$^-$	1.23 (0.78–1.94)	0.36	1.17 (0.73–1.88)	0.49
CD34$^+$_high/MetS$^+$	1.74 (0.99–3.05)	0.051	1.64 (0.92–2.93)	0.08
CD34$^+$_low/MetS$^+$	5.19 (2.72–9.90)	**<0.0001**	3.98 (2.01–7.87)	**<0.0001**
Neutrophils_low/MetS$^-$	Reference		Reference	
Neutrophils_low/MetS$^+$	2.36 (1.21–4.58)	**0.011**	2.16 (1.09–4.28)	**0.026**
Neutrophils_high/MetS$^-$	2.38 (1.49–3.81)	**<0.0001**	2.03 (1.25–3.29)	**0.004**
Neutrophils_high/MetS$^+$	4.54 (2.67–7.74)	**<0.0001**	3.58 (2.03–6.37)	**<0.0001**

*Adjusted for age, gender and hs-CRP.

Compared with controls, subjects with VTE showed a marginally significant reduction in the CD34$^+$ cell count ($P = 0.066$). However, when we stratified the patients according to the type of VTE (provoked and unprovoked), we observed a significantly lower CD34$^+$ cell count in the group of subjects with unprovoked VTE compared with both provoked VTE ($P = 0.004$) and controls ($P = 0.003$) (Figure 1). Instead, patients with provoked VTE and controls showed similar levels of circulating CD34$^+$ cells ($P = 0.49$). Demographic and laboratory characteristics of subjects with provoked and unprovoked events were similar except for the CD34$^+$ cell count and the prevalence of active/previous smokers [Figure 1 and Supplementary Table S2 (at http://www.clinsci.org/cs/125/cs1250211add.htm)]. In addition, the temporal distance between CD34$^+$ cell/neutrophil measurement and VTE was not different between these two groups of patients (provoked, 65.31 $\pm$ 40.6 months; unprovoked, 69.78 $\pm$ 44.9 months; $P = 0.47$). Moreover, we looked for a linear relationship between CD34$^+$/neutrophil cell count and the temporal distance from the VTE event, which was not significant (neutrophils: $r = -0.033$, $P = 0.68$; CD34$^+$ cells: $r = -0.084$, $P = 0.30$). Differences in CD34$^+$ levels between provoked and unprovoked VTE remained significant even after adjustment for age, gender, the presence of MetS, neutrophil count, hs-CRP, smoking habit, use of antihypertensive treatments/statins/aspirin and distance from the event (CD34$^+$ cells, $P = 0.009$). We also calculated the level of CD34$^+$ cells in different subgroups of VTE and observed a significant reduction in the cell count among patients with proximal DVT compared with those with history of distal events ($P = 0.001$). No differences were observed in CD34$^+$ cell count among patients grouped according to the presence of PE, thrombophilia, history of recurrence and use of VKAs (Supplementary Figure S1 at http://www.clinsci.org/cs/125/cs1250211add.htm).

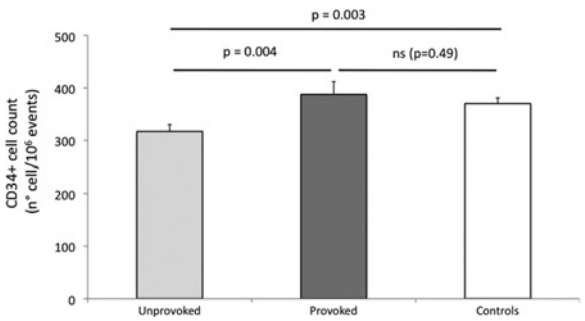

Figure 1 Levels of circulating CD34$^+$ cells in subjects grouped according to the type of VTE

Unprovoked, $n = 161$; provoked, $n = 79$; controls, $n = 240$; ANOVA, $P = 0.003$, CD34$^+$ cell counts are expressed as means + S.E.M.

Interaction of circulating CD34$^+$ cells/neutrophils with MetS and VTE risk

To better discriminate the level of interaction between neutrophils, CD34$^+$ cells and MetS in determining VTE risk, we classified both cases and controls into four groups according to the presence/absence of MetS (MetS$^+$ or MetS$^-$) and the level (high/low) of both CD34$^+$ cell count and neutrophils. The analysis was conducted by using the group neutrophils_low/MetS$^-$ and the group CD34$^+$_high/MetS$^-$ as reference. When we looked at the interaction between MetS and neutrophils, we observed that both of the neutrophils_low/MetS$^+$ and neutrophils_high/MetS$^-$ groups showed a significant increase in VTE risk compared with the reference group (Table 3). Nevertheless, the highest increase in OR for VTE was observed among subjects showing both high neutrophil levels and the presence of MetS (neutrophils_high/MetS$^+$) (Table 3). All of these findings remained significant even after adjustment for age, gender and hs-CRP. The analysis of the interaction between CD34$^+$ cell count and MetS showed that only subjects belonging to the CD34$^+$_low/MetS$^+$ group displayed a significant increase in VTE risk compared with the CD34$^+$_high/MetS$^-$ group, considered as reference. In particular, the crude and adjusted OR for VTE in the group CD34$^+$_low/MetS$^+$ as compared with the group CD34$^+$_high/MetS$^-$ were 5.19 (95% CI 2.72–9.90, $P < 0.0001$) and 3.98 (95% CI 2.01–7.87, $P < 0.0001$) respectively (Table 3). This effect was not modified by taking into account the neutrophil levels (see Supplementary Table S3 at http://www.clinsci.org/cs/125/cs1250211add.htm). To further analyse the synergy effect between low CD34$^+$ cell count, high neutrophil levels and MetS for VTE risk we calculated the Rothman SI (a value significantly >1 implies synergism as a more-than-additive interaction, whereas a value significantly <1 implies antagonism as a less-than-additive interaction). In particular, the SI value for high neutrophils and MetS was 0.866 (0.608–1.234; $P = 0.44$) suggesting that the interaction between these

two factors for VTE does not depart from an additive model. In contrast, the SI value for low $CD34^+$ cell count and MetS was 2.016 (1.303–3.091; $P = 0.0016$) indicative of a synergism between these factors in generating VTE risk (combination of low $CD34^+$ cell count and the presence of MetS provides a greater risk than the sum of their individual effects).

DISCUSSION

In the present study, we demonstrate for the first time that patients with a history of unprovoked VTE had reduced circulating levels of $CD34^+$ progenitor cells compared with either subjects with provoked VTE or control individuals. In addition, we observed that patients with any type of VTE displayed an increase in neutrophil levels compared with controls. This finding remains strongly significant even after adjustment for hs-CRP, a sensitive marker of subclinical inflammation. Finally, both reduced $CD34^+$ blood cell count and high neutrophil levels significantly interact with the presence of MetS in raising VTE risk.

Our findings about the association of MetS with VTE risk are in agreement with data from previous clinical studies. In particular, the 1.96 estimated OR for VTE observed among patients with MetS is in line with the 1.94 and 2.20 ORs previously described [9,11]. Among the different components of MetS, central obesity emerged in our population as the strongest contributor to VTE risk. A significant association was also observed for elevated blood glucose and TAG, whereas high BP and reduced HDL-C showed a weak correlation with VTE risk. The central role played by abdominal obesity in predicting VTE risk has also been confirmed in longitudinal studies [22,23]. In contrast, these prospective investigations reported a limited contribution of other MetS components for the prediction of VTE events [22,23]. On the whole, these findings suggest that the presence of MetS is not sufficient in driving the risk for VTE and other superimposing risk factors could contribute to the onset of venous thromboembolic events.

Several clinical studies showed a significant association between low circulating number of $CD34^+$ cells and the risk of future atherosclerotic events [15,17]. These progenitor cells have been shown to contribute in preserving normal vascular homoeostasis and actively participate in vasculogenesis and endothelial healing [24]. In addition, animal and clinical studies have also shown beneficial effects of these cells in the prevention of arterial thrombosis [25,26]. These protective properties can be explained not only by the enhanced re-endothelialization and neovascularization promoted by the progenitor cells, but also by the presence of inhibitory effects on platelets aggregation and thrombus formation [16]. However, the role played by circulating $CD34^+$ cells in the venous system and during VTE pathogenesis has so far been poorly investigated. Few reports indicated a possible contribution of these cellular elements in the lyses of the thrombi. In fact, $CD34^+$ cell recruitment has been observed within resolving venous thrombi [27] and injection of endothelial progenitor cells in animal models of venous thrombosis is followed by accelerated vascular recanalization [28]. Of interest, in our population, the number of circulating $CD34^+$ cells was significantly reduced only among subjects with a history of unprovoked VTE, whereas patients with provoked VTE and control subjects displayed similar $CD34^+$ levels. This finding suggests that a reduction in the progenitor blood cell count might have a negative impact on the venous system in a way similar to what has been described in the arterial system. In particular, it could be postulated that impaired vascular reparative processes and/or defective mechanisms of thrombus resolving might be present in subjects with a reduced $CD34^+$ blood cell count, thus generating a permissive soil for thrombotic events. Of interest, when we grouped the study population on the basis of the presence/absence of MetS and $CD34^+$ levels, we observed that the sum of MetS and low $CD34^+$ circulating cells exerts a synergistic effect in generating VTE risk (Table 3). This additive effect seems to be unrelated to the neutrophil levels (Supplementary Table S3). Analysis of $CD34^+$ levels in the different subgroups of patients with VTE showed that the cell count was significantly reduced among subjects with the history of proximal DVT compared with those with distal events. No significant differences were observed on the basis of the presence/absence of PE, history of recurrence and thrombophilia. Additional prospective investigations are needed to establish whether the $CD34^+$ blood cell count predicts the risk of future VTE events and could be used to identify subjects carrying additional VTE risk.

Interestingly a number of recent basic science studies highlighted the centrality of neutrophil activation in the early phase of thrombus formation [29]. In particular, interaction of neutrophils with endothelial cells through adhesion molecules (such as P-selectin) and the release by the cells of pro-thrombotic activators (such as neutrophil extracellular traps) are now considered important pathogenetic steps of venous thrombogenesis [30,31]. In addition, recent findings suggest that neutrophils, after binding to injured endothelial cells, represent a major source of tissue factor needed for arterial thrombus formation [32]. Moreover, serine proteinases derived from neutrophils, in concert with externalized nucleosomes, can promote thrombotic occlusion of large vessels [33]. In line with these observations, clinical studies showed that, among the different WBC subtypes, high neutrophil levels displayed the greater predictive value for future atherothrombotic events [34]. However, despite these data, the importance of neutrophil levels and their correlation with other pro-thrombotic factors in generating VTE risk has so far been poorly investigated. Of interest, in our population a striking increase in circulating neutrophil levels was observed in VTE cases compared with controls. No differences between the two groups were seen for lymphocytes, basophils and eosinophils, while the modest increase in monocytes did not stand adjustment for potential confounders. In contrast, the increase in neutrophil count in VTE remains statistically significant even after adjustment for age, gender, $CD34^+$ count, hs-CRP levels, and the presence of MetS and each of its components. This independent predictive effect suggests that a mild elevation in neutrophil count, even within the normal range, should be considered in itself as a risk factor for VTE. Previous studies investigating the involvement of neutrophils in thrombogenesis underscored the active role played by these cells during thrombus resolution [35]. These findings should not be considered conflicting or contradictory with the

more recent demonstrations of neutrophil participation in the early stage of thrombosis. In contrast, these basic science data coupled with our findings propel towards the conduction of additional clinical studies aimed at clarifying the role played by neutrophil phenotypic profiles and activation state in the pathogenesis of thrombotic events. Nevertheless, a positive correlation between features of MetS and WBC count has been observed in other clinical settings [19], and neutrophils have been recently implicated in the generation of insulin resistance associated with obesity [36]. Whether neutrophils might be the link between abdominal obesity and VTE risk certainly represents an interesting matter of investigation. In line with this possibility, differently from CD34+ cell count, neutrophil levels did not display synergism with MetS and were similar between unprovoked and provoked VTE. Moreover, recent studies also demonstrated the importance of neutrophil activation for the formation of thrombi associated with venous stasis, one of the most frequent conditions of provoked VTE [31].

Major limitations for the interpretation of our findings are the retrospective nature of the study and the heterogeneous distance of VTE events from clinical and biochemical measurements. In particular, the design of the study does not allow establishing a causal relationship between low CD34+ cells/high neutrophils and VTE pathogenesis. Only a prospective investigation would provide a more comprehensive pathophysiological explanation on the role played by progenitor cells and neutrophil levels during thrombogenesis. Nevertheless, our findings about CD34+ cells are in line with prospective data showing the negative predictive role of these cells for future cardiovascular events [15]. Under this point of view, our observation might offer an interesting pathophysiological explanation for the link between unprovoked VTE events and the risk of atherosclerosis [3]. Data about neutrophils are more difficult to interpret and might be in fact expression of a persistent 'low grade' inflammation induced by the previous VTE event. However, as mentioned above, the association between high neutrophils and VTE risk remains strongly significant even after adjustment for markers of chronic inflammation (such as hs-CRP) and all of the MetS features. Moreover, it should also be emphasized that we excluded from the study all of the subjects with evidence of local/systemic inflammatory disease that the WBC levels were within the normal range and that among the different WBC subpopulations only neutrophils remain strongly associated with VTE risk. Finally, the CD34+ cell and neutrophil findings were not significantly correlated with the temporal distance between VTE event and the enrolment in the study.

In conclusion, the results of the present study have shown that low CD34+ blood cell count and high circulating neutrophils significantly interplay with the presence of MetS in raising the risk for VTE events.

CLINICAL PERSPECTIVES

- The risk of atherosclerosis is increased among subjects with history of VTE and features of MetS have been implicated in VTE risk. Previous studies showed that low circulating levels of progenitor cells are associated with increased risk of atherosclerosis, but no information is available about their role in VTE.
- In the present study, we showed that circulating levels of progenitor cells are significantly reduced in patients with unprovoked VTE and that high neutrophil levels are associated with increased VTE risk. Both low progenitor cells and high neutrophil levels interplay with features of MetS in raising the VTE risk.
- These findings can offer the opportunity to identify patients with elevated risk of new/recurrent VTE.

AUTHOR CONTRIBUTION

Marcello Rattazzi , Sabina Villalta, Gian Paolo Fadini and Paolo Pauletto designed the study, supervised the collection of the data and wrote the paper. Silvia Galliazzo, Roberta Buso, Elena Callegari, Alessandra Sponchiado and Valeria Pagliara recruited the patients and collected clinical data. Elisabetta Faggin, Laura Del Pup, Elisa Bertacco, Elena Seganfreddo and Livio Caberlotto performed laboratory and flow cytometry analyses. Massimo Puato and Gianluigi Scannapieco performed statistical analyses and wrote and edited the paper.

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REFERENCES

1 Spencer, F. A., Ginsberg, J. S., Chong, A. and Alter, D. A. (2008) The relationship between unprovoked venous thromboembolism, age, and acute myocardial infarction. J. Thromb. Haemostasis **6**, 1507–1513
2 Prandoni, P., Bilora, F., Marchiori, A., Bernardi, E., Petrobelli, F., Lensing, A. W., Prins, M. H. and Girolami, A. (2003) An association between atherosclerosis and venous thrombosis. N. Engl. J. Med. **348**, 1435–1441
3 Sorensen, H. T., Horvath-Puho, E., Pedersen, L., Baron, J. A. and Prandoni, P. (2007) Venous thromboembolism and subsequent hospitalisation due to acute arterial cardiovascular events: a 20-year cohort study. Lancet **370**, 1773–1779
4 Franchini, M. and Mannucci, P. M. (2008) Venous and arterial thrombosis: different sides of the same coin? Eur. J. Intern. Med. **19**, 476–481
5 McNeill, A. M., Rosamond, W. D., Girman, C. J., Golden, S. H., Schmidt, M. I., East, H. E., Ballantyne, C. M. and Heiss, G. (2005) The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. Diabetes Care **28**, 385–390
6 Ageno, W., Becattini, C., Brighton, T., Selby, R. and Kamphuisen, P. W. (2008) Cardiovascular risk factors and venous thromboembolism: a meta-analysis. Circulation **117**, 93–102
7 Deguchi, H., Pecheniuk, N. M., Elias, D. J., Averell, P. M. and Griffin, J. H. (2005) High-density lipoprotein deficiency and dyslipoproteinemia associated with venous thrombosis in men. Circulation **112**, 893–899
8 Doggen, C. J., Smith, N. L., Lemaitre, R. N., Heckbert, S. R., Rosendaal, F. R. and Psaty, B. M. (2004) Serum lipid levels and the risk of venous thrombosis. Arterioscler. Thromb. Vasc. Biol. **24**, 1970–1975

9 Ageno, W., Prandoni, P., Romualdi, E., Ghirarduzzi, A., Dentali, F., Pesavento, R., Crowther, M. and Venco, A. (2006) The metabolic syndrome and the risk of venous thrombosis: a case-control study. J. Thromb. Haemostasis **4**, 1914–1918
10 Franchini, M., Targher, G., Montagnana, M. and Lippi, G. (2008) The metabolic syndrome and the risk of arterial and venous thrombosis. Thromb. Res. **122**, 727–735
11 Ay, C., Tengler, T., Vormittag, R., Simanek, R., Dorda, W., Vukovich, T. and Pabinger, I. (2007) Venous thromboembolism – a manifestation of the metabolic syndrome. Haematologica **92**, 374–380
12 Steinmetz, M., Nickenig, G. and Werner, N. (2010) Endothelial-regenerating cells: an expanding universe. Hypertension **55**, 593–599
13 Fadini, G. P., Rattazzi, M., Matsumoto, T., Asahara, T. and Khosla, S. (2012) Emerging role of circulating calcifying cells in the bone-vascular axis. Circulation **125**, 2772–2781
14 Asahara, T., Kawamoto, A. and Masuda, H. (2011) Concise review: circulating endothelial progenitor cells for vascular medicine. Stem Cells **29**, 1650–1655
15 Fadini, G. P., de Kreutzenberg, S. V., Coracina, A., Baesso, I., Agostini, C., Tiengo, A. and Avogaro, A. (2006) Circulating $CD34^+$ cells, metabolic syndrome, and cardiovascular risk. Eur. Heart J. **27**, 2247–2255
16 Abou-Saleh, H., Yacoub, D., Theoret, J. F., Gillis, M. A., Neagoe, P. E., Labarthe, B., Theroux, P., Sirois, M. G., Tabrizian, M., Thorin, E. et al. (2009) Endothelial progenitor cells bind and inhibit platelet function and thrombus formation. Circulation **120**, 2230–2239
17 Fadini, G. P., de Kreutzenberg, S., Agostini, C., Boscaro, E., Tiengo, A., Dimmeler, S. and Avogaro, A. (2009) Low $CD34^+$ cell count and metabolic syndrome synergistically increase the risk of adverse outcomes. Atherosclerosis **207**, 213–219
18 Chen, W., Srinivasan, S. R., Xu, J. and Berenson, G. S. (2010) Black-white divergence in the relation of white blood cell count to metabolic syndrome in preadolescents, adolescents, and young adults: the Bogalusa Heart Study. Diabetes Care **33**, 2474–2476
19 Fadini, G. P., Marcuzzo, G., Marescotti, M. C., de Kreutzenberg, S. V. and Avogaro, A. (2012) Elevated white blood cell count is associated with prevalence and development of the metabolic syndrome and its components in the general population. Acta Diabetol. **49**, 445–451
20 Gregor, M. F. and Hotamisligil, G. S. (2011) Inflammatory mechanisms in obesity. Annu. Rev. Immunol. **29**, 415–445
21 Ashwell, M., Chinn, S., Stalley, S. and Garrow, J. S. (1982) Female fat distribution – a simple classification based on two circumference measurements. Int. J. Obes. **6**, 143–152
22 Borch, K. H., Braekkan, S. K., Mathiesen, E. B., Njolstad, I., Wilsgaard, T., Stormer, J. and Hansen, J. B. (2009) Abdominal obesity is essential for the risk of venous thromboembolism in the metabolic syndrome: the Tromso study. J. Thromb. Haemostasis **7**, 739–745
23 Steffen, L. M., Cushman, M., Peacock, J. M., Heckbert, S. R., Jacobs, Jr, D. R., Rosamond, W. D. and Folsom, A. R. (2009) Metabolic syndrome and risk of venous thromboembolism: longitudinal investigation of thromboembolism etiology. J. Thromb. Haemostasis **7**, 746–751
24 Hristov, M. and Weber, C. (2008) Endothelial progenitor cells in vascular repair and remodeling. Pharmacol. Res. **58**, 148–151
25 Kaushal, S., Amiel, G. E., Guleserian, K. J., Shapira, O. M., Perry, T., Sutherland, F. W., Rabkin, E., Moran, A. M., Schoen, F. J., Atala, A. et al. (2001) Functional small-diameter neovessels created using endothelial progenitor cells expanded *ex vivo*. Nat. Med. **7**, 1035–1040
26 Co, M., Tay, E., Lee, C. H., Poh, K. K., Low, A., Lim, J., Lim, I. H., Lim, Y. T. and Tan, H. C. (2008) Use of endothelial progenitor cell capture stent (Genous Bio-Engineered R Stent) during primary percutaneous coronary intervention in acute myocardial infarction: intermediate- to long-term clinical follow-up. Am. Heart J. **155**, 128–132
27 Modarai, B., Burnand, K. G., Sawyer, B. and Smith, A. (2005) Endothelial progenitor cells are recruited into resolving venous thrombi. Circulation **111**, 2645–2653
28 Meng, Q., Li, X., Yu, X., Lei, F., Jiang, K. and Li, C. (2011) Transplantation of *ex vivo* expanded bone marrow-derived endothelial progenitor cells enhances chronic venous thrombus resolution and recanalization. Clin. Appl. Thromb./Hemostasis **17**, E196–E201
29 Fuchs, T. A., Brill, A. and Wagner, D. D. (2012) Neutrophil Extracellular Trap (NET) impact on deep vein thrombosis. Arterioscler. Thromb. Vasc. Biol. **32**, 1777–1783
30 Brill, A., Fuchs, T. A., Savchenko, A. S., Thomas, G. M., Martinod, K., De Meyer, S. F., Bhandari, A. A. and Wagner, D. D. (2012) Neutrophil extracellular traps promote deep vein thrombosis in mice. J. Thromb. Haemostasis **10**, 136–144
31 von Bruhl, M. L., Stark, K., Steinhart, A., Chandraratne, S., Konrad, I., Lorenz, M., Khandoga, A., Tirniceriu, A., Coletti, R., Kollnberger, M. et al. (2012) Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice *in vivo*. J. Exp. Med. **209**, 819–835
32 Darbousset, R., Thomas, G. M., Mezouar, S., Frere, C., Bonier, R., Mackman, N., Renne, T., Dignat-George, F., Dubois, C. and Panicot-Dubois, L. (2012) Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. Blood **120**, 2133–2143
33 Massberg, S., Grahl, L., von Bruehl, M. L., Manukyan, D., Pfeiler, S., Goosmann, C., Brinkmann, V., Lorenz, M., Bidzhekov, K., Khandagale, A. B. et al. (2010) Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat. Med. **16**, 887–896
34 Horne, B. D., Anderson, J. L., John, J. M., Weaver, A., Bair, T. L., Jensen, K. R., Renlund, D. G. and Muhlestein, J. B. (2005) Which white blood cell subtypes predict increased cardiovascular risk? J. Am. Coll. Cardiol. **45**, 1638–1643
35 Varma, M. R., Varga, A. J., Knipp, B. S., Sukheepod, P., Upchurch, G. R., Kunkel, S. L., Wakefield, T. W. and Henke, P. K. (2003) Neutropenia impairs venous thrombosis resolution in the rat. J. Vasc. Surg. **38**, 1090–1098
36 Talukdar, S., Oh, D. Y., Bandyopadhyay, G., Li, D., Xu, J., McNelis, J., Lu, M., Li, P., Yan, Q., Zhu, Y. et al. (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat. Med. **18**, 1407–1412

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online data

Original Paper

SUPPLEMENTARY ONLINE DATA

Low CD34$^+$ cells, high neutrophils and the metabolic syndrome are associated with an increased risk of venous thromboembolism

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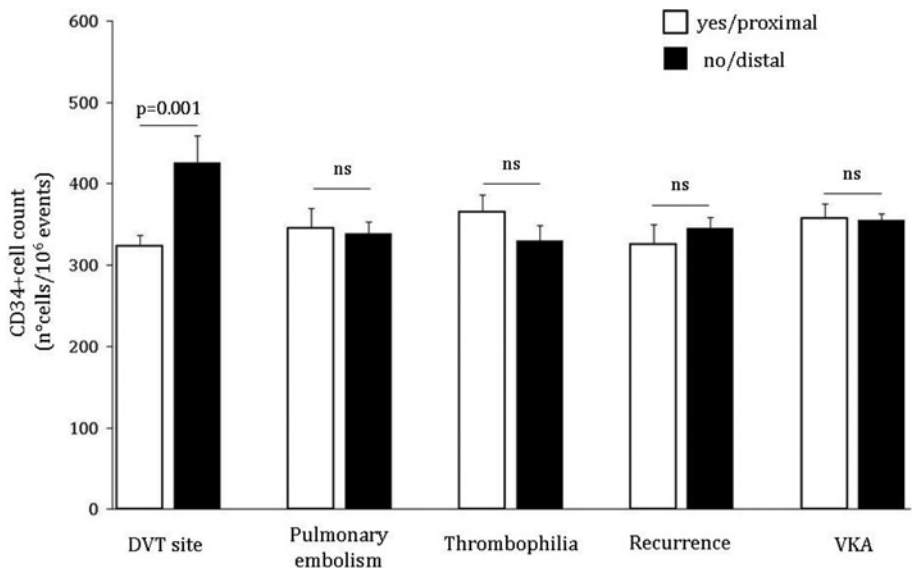

Figure S1 **CD34$^+$ cell distribution according to VTE characteristics**
ns, not significant.

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Table S1 Results of multivariate logistic regression analysis for VTE risk

	β coefficient	OR (95% CI)	*P* value
Model 1			
MetS	0.546	1.72 (1.11–2.68)	**0.015**
Age	0.004	1.00 (0.99–1.01)	0.57
Male gender	0.165	1.17 (0.78–1.76)	0.42
Neutrophils	0.000	1.00 (1.00–1.01)	**<0.0001**
Monocytes	−0.001	0.99 (0.99–1.00)	0.21
Active/previous smokers	0.264	1.30 (0.88–1.92)	0.18
hs-CRP	0.747	2.11 (1.13–3.94)	**0.019**
Model 2			
Age	−0.002	0.99 (0.98–1.01)	0.84
Male gender	−0.052	0.94 (0.61–1.46)	0.81
Active/previous smokers	0.269	1.30 (0.87–1.95)	0.18
Neutrophils	0.000	1.00 (1.00–1.01)	**<0.0001**
hs-CRP	0.712	2.03 (1.09–3.77)	**0.024**
SBP $\geqslant$130 mmHg or DBP $\geqslant$85 mmHg	0.206	1.22 (0.73–2.06)	0.43
HDL-C $\leqslant$40 mg/dl male or $\leqslant$50 mg/dl female	−0.395	0.67 (0.36–1.23)	0.20
TAG $\geqslant$150 mg/dl	0.465	1.59 (0.90–2.79)	0.10
Glycaemia $\geqslant$100 mg/dl	0.462	1.58 (1.01–2.48)	**0.043**
Waist circumferences $\geqslant$102 cm male or $\geqslant$88 cm female	0.477	1.61 (1.06–2.43)	**0.024**

Table S2 Characteristics of the population according to VTE type

Values are means (S.D.), medians (interquartile range) or numbers (percentage). eGFR, estimated glomerular filtration rate.

Parameter	Unprovoked	Provoked	*P* value
n	161	79	
Age (years)	57.2 (14.5)	56.8 (14.3)	0.98
Male gender (*n*)	88 (54.7%)	36 (45.6%)	0.18
BMI (kg/m^2)	28.0 (4.9)	26.9 (4.7)	0.19
Waist circumference (cm)	101.86 (12.2)	100.5 (12.8)	0.64
Active/previous smokers (*n*)	89 (55.3%)	27 (34.2%)	**0.002**
History of atherosclerotic events (*n*)	7 (4.3%)	5 (6.3%)	0.26
SBP (mmHg)	134.3 (18.6)	133.1 (19.9)	0.65
DBP (mmHg)	88.2 (11.6)	87.7 (10.4)	0.96
eGFR male (ml/min per 1.73 m^2)	79.8 (23.0)	86.6 (21.6)	0.41
eGFR female (ml/min per 1.73 m^2)	82.4 (21.1)	82.6 (17.7)	0.96
Total cholesterol (mg/dl)	211.5 (42.5)	204.5 (36.5)	0.24
HDL-C (mg/dl)	58.8 (16.2)	57.6 (14)	0.98
LDL-C (mg/dl)	129.1 (39.7)	122.4 (33.9)	0.21
TAG (mg/dl)	100 (69.5–146)	103 (77–142.5)	0.85
Glycaemia (mg/dl)	94.5 (87.25–106)	95 (89–103)	0.52
Diabetes (*n*)	18 (11.1%)	5 (6.3%)	0.36
Arterial hypertension (*n*)	101 (62.7%)	45 (56.9%)	0.72
Therapy with statins (*n*)	27 (16.7%)	11 (13.9%)	0.87
Antiplatelet therapy (*n*)	15 (9.3%)	9 (11.3%)	0.39
Oral anticoagulants (*n*)	77 (47.8)	40 (50.6%)	0.39
hs-CRP (mg/dl)	0.20 (0.10–0.40)	0.11 (0.08–0.40)	0.31
Distance from VTE event (months)	69.78 (44.9)	65.31 (40.6)	0.47
MetS (*n*)	62 (38.5%)	30 (37.9%)	0.98
Platelets (*n*/mm^3)	254 906.8 (69 158.3)	262 417.7 (57 998.3)	0.40
Haemoglobin (mg/dl)	14.3 (1.5)	14.1 (1.3)	0.59
Leucocytes (*n*/mm^3)	6692.4 (1872.3)	6506.4 (1527.8)	0.44
Neutrophils (*n*/mm^3)	3858.5 (1476.4)	3677.5 (1348.9)	0.36
Lymphocytes (*n*/mm^3)	2127.2 (653.9)	2137.4 (597.2)	0.90
Monocytes (*n*/mm^3)	457.1 (141.8)	449.7 (139.6)	0.70
Eosinophils (*n*/mm^3)	218.4 (210.2)	221.1 (164.7)	0.92
Basophils (*n*/mm^3)	43.7 (32.7)	47.4 (32.4)	0.40

Table S3 OR for VTE

Parameter	Adjusted OR (95% CI)*	*P* value
$CD34^+$_high/Neutrophils_low/$MetS^-$	Reference	
$CD34^+$_high/Neutrophils_high/$MetS^-$	2.36 (1.19–4.66)	**0.013**
$CD34^+$_low/Neutrophils_low/$MetS^-$	1.20 (0.66–2.19)	0.54
$CD34^+$_low/Neutrophils_high/$MetS^-$	2.16 (1.19–3.93)	**0.011**
$CD34^+$_high/Neutrophils_high/$MetS^+$	2.57 (1.27–5.21)	**0.009**
$CD34^+$_high/Neutrophils_low/$MetS^+$	1.13 (0.45–2.86)	0.78
$CD34^+$_low/Neutrophils_low/$MetS^+$	4.27 (1.50–12.12)	**0.006**
$CD34^+$_low/Neutrophils_high/$MetS^+$	5.47 (2.36–12.65)	**<0.0001**

*Adjusted for age, gender and hs-CRP.

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