Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk

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Aims Circulating progenitor cells are believed to participate in cardiovascular (CV) homeostasis and their exhaustion has been linked to CV damage. As general agreement on their characterization is lacking, this work was carried out to assess the relationships between different antigenic profiles of progenitor cells and CV risk, with special regard to metabolic syndrome (MetSyn).

Methods and results CD34, CD133, and KDR were used to quantify circulating progenitors in 214 subjects at different levels of CV risk. In a cross-analysis of six different cell subtypes (CD34⁺, CD133⁺, CD34⁺CD133⁺, CD34⁺KDR⁺, CD133⁺KDR⁺, and CD34⁺CD133⁺KDR⁺), CD34⁺ progenitors showed the best correlation with CV parameters and risk estimates. Components of the MetSyn were all characterized by reduction of CD34⁺ cells and acted synergistically in decreasing CD34⁺ cell count. Moreover, CD34⁺ cell count demonstrated a high performance in detecting high CV risk.

Conclusion These data demonstrate that CD34 identifies progenitor cells linked to CV risk, showing a close negative correlation between CD34⁺ cells and CV risk, as well as a synergic detrimental effect of clustered metabolic components. Progenitor cell count may be used as a surrogate marker of CV risk, whereas extensive antigenic characterization may not be useful for this purpose.

Introduction

Cardiovascular diseases (CVD) are the leading cause of death in western countries and risk assessment has become mandatory in the adult population.¹ Over the past decades, it has become clear that patients with a given combination of risk factors, clustered in the so-called metabolic syndrome (MetSyn), are more prone to develop CVD.² Besides the classical risk factors, novel biomarkers have recently received much attention³ and an extreme plasticity is being attributed to circulating progenitor cells. Classical theories affirm that each organ and tissue harvests a subset of relatively immature resident cells, which are quiescent in normal conditions, but are capable of re-entering into the cell cycle when organ homeostasis is perturbed. This view has been challenged by a novel paradigm, by which bone marrow is a reservoir of immature cells which, once in the bloodstream, participate in regeneration and repair of many tissues, not limited to the haematopoietic lineages.⁴

In this scenario, a special interest has developed in the relationships between progenitor cells and CVD. So far, endothelial progenitor cells (EPCs) are the most studied vascular progenitors.⁵ Levels of circulating EPCs, defined as CD34⁺KDR⁺ cells, are reduced in the presence of classical risk factors for CVD, such as diabetes, smoking, hypercholesterolaemia, and hypertension,⁶–⁸ and in the setting of the three common presentations of established CVD: coronary artery disease (CAD), cerebrovascular disease, and peripheral artery disease.⁶,⁷,⁹,¹⁰ Moreover, even among healthy subjects, a negative correlation has been reported between progenitor cells and endothelial function.¹¹ Consistently, CD34⁺KDR⁺ cell count correlates with cumulative indexes of CV risk² and represents an independent predictor of atherosclerosis progression and CV events.¹²,¹³ Unfortunately, no single definition of CV progenitor cells exists, and it is unknown which is the best antigenic profile to identify progenitor cells linked to CV risk.¹⁴

Therefore, the aim of the present study was to assess the relationships of different circulating progenitor cells, identified using three markers and six antigenic combinations, with CV parameters in a sample of subjects covering a wide spectrum of CV risk. Our results indicate that among all antigenic combinations, CD34⁺ progenitor cells best reflect CV risk and are synergistically reduced in the presence of MetSyn and its components.

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Methods

Patients

The study was approved by the local Ethics Committee. A sample size of about 200 subjects was used to detect relevant correlations between progenitor cell count and CV risk, with the following assumptions based on previous data: type I error (α) of 0.01, power (1−β) of 99%, SD of the regression errors (σ) of 160, SD of independent variable (νs) of 19, and slope of the regression line (λ) of 3 (equal to a correlation coefficient ρ of 0.34). The same sample size would allow detection of a weaker correlation (λ = 1.7; ρ = 0.19) with typical α = 0.05 and β = 0.20. Selection and inclusion were designed to maximize the range of CV risk covered by the study sample. For this purpose, healthy subjects (n = 114), reflecting a middle-aged general population, were recruited voluntarily from a local community of office employees on the basis of an agreement with the University of Padova. Subjects with higher prevalence of risk factors were recruited as follows: 197 patients consecutively presenting at our outpatient and inpatient clinic for metabolic and related disorders (diabetes, hypertension, dyslipidaemia, and obesity) were initially assessed; 103 were deemed eligible because they did not meet exclusion criteria, and 100 were finally included in the study, 62% of which had an established CVD and 38% were classified as ‘at risk’ due to their risk profile. No patient refused to give informed consent, and three patients were dropped out because of unavailable measurements. The following data were recorded: age, gender, body mass index (BMI), waist circumference, fasting blood glucose, systolic and diastolic blood pressures, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), and triglyceride concentrations. CVD was defined as CAD (unambiguous findings suggestive of past myocardial infarction on a resting electrocardiogram or a positive myocardial ischaemia stress-test or evidence of a significant coronary artery stenosis), cerebrovascular disease (defined as significant carotid artery stenosis on a Doppler ultrasonography or a carotid angiography), or peripheral arterial disease (defined as evidence of severe atherosclerosis obliteration using Doppler ultrasonography, ankle-brachial index, or angiography). The following predetermined exclusion criteria applied to both healthy subjects and patients: subject refusal, acute infections or illnesses, neoplasms, haemodialysis, recent (within 3 months) myocardial infarction, surgery or endovascular intervention, unstable angina, uncontrolled hypertension or hyperglycaemia, and immunological diseases.

Risk factors and estimates of CV risk

The following risk factors were considered: diabetes mellitus (defined by fasting or 2-h post-challenge glucose levels), current smoking habit (of one or more cigarette per day), obesity (defined as a BMI > 30 kg/m² or a waist circumference > 102 cm in men or 88 cm in women), hypertension (defined as a systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg or the use of hypotensive drugs), hyperlipidaemia (defined as a total cholesterol > 200 mg/dL or a triglyceride concentration > 150 mg/dL or the use of hypolipidaemic drugs), family history (defined as CVD in one or more first-degree relatives), and age over 50. The 10 years of predicted CV risk according to the Framingham study was calculated as previously described. We calculated also the 10-year risk according to the Italian risk-assessment program (accessible at the url: http://www.cuore.iss.it/sopra/calc-rischio_en.asp). For the criteria of the MetSyn, the definitions of ATP-III were used. Patients were classified according to the number of criteria they fulfilled. Predicted levels of progenitor cells according to the number of criteria of the MetSyn were derived as follows: the effect of each single component on progenitor cell count was calculated as cell variation in subjects with one component only vs. control subjects; the predicted cell count in subjects with one to five components was derived applying the mean %reduction to the mean count of control subjects as if the presence of one to five components was additive. For example, the predicted mean progenitor cell reduction in patients with obesity and hypertension was calculated as mean cell count in control subjects – (%reduction in patients with obesity alone + %reduction in patients with hypertension alone). The analysis was repeated for all combinations of two, three, four, and five components to obtain the mean %reduction associated with two to five components.

Progenitor cells quantification in peripheral blood

Fasting blood samples were processed after 1-2 h. Peripheral blood progenitor cells were analysed for the expression of surface antigens with direct two- or three-colour flow cytometry (FACS Calibur, Becton Dickinson) as previously reported. Briefly, before staining with specific monoclonal antibodies, cells were treated with fetal calf serum for 10 min, then the samples were washed with buffer containing phosphate-buffered saline and 0.5% bovine albumin. Then, 150 µL of peripheral blood was stained with 10 µL of FITC-conjugated anti-human CD34 mAb (Becton Dickinson), 10 µL of PE-conjugated anti-human KDR mAb (R&D Systems), and 10 µL of APC-conjugated anti-CD133 mAb (Miltenyi Biotech). Control isotypes IgG1 and IgG2A Abs were obtained from Becton Dickinson. The frequency of peripheral blood cells positive for these reagents was determined by a 2D side scatter-fluorescence dot-plot analysis, after appropriate gating, stained with the different reagents. We gated CD34+ or CD133+ peripheral blood cells in the mononuclear cell fraction and then examined the resulting population for the dual expression of KDR. At the intersection of the CD34 and CD133 gates, we identified CD34+CD133+ cells, whereas total KDR+ mononuclear cells were identified separately as cells with high KDR expression and low side scatter. Triple positive cells were identified by the dual expression of KDR and CD34 or CD134 in the CD133 or CD34 gates, respectively (Figure 1). In all subjects, expression of CD34 and KDR was studied, whereas the complete assay, including assessment of CD133 expression, was performed in a subset of 136 (64% of total). For the FACS analysis, 5 × 10⁵ cells were acquired and scored using a FACS Calibur analyzer (Becton Dickinson). Data were processed using the Macintosh CELLQuest software program (Becton Dickinson). The instrument set-up was optimized daily by analysing the expression of peripheral blood lymphocytes labelled with anti-CD4 FITC/CD8 PE/CD3 PE/Cy5/CD45 APC four-colour combination. The same trained operator, who was blind to the clinical status of the patients, performed all the tests throughout the study. Measures were repeated twice in two separate blood samples from 21 subjects (9.8% of total). To assess the test–retest reliability, we obtained mean-centred coefficients of variation (CV) and intra-class correlation coefficients calculated using an absolute agreement definition and a two-way random effects model (Supplementary Material, Table 15).

Statistical analysis

Data are expressed as mean ± SD unless otherwise noted. The results from flow cytometry are expressed as number of cells/10⁵ events. Differences between two or more groups were evaluated by two-sided Student's t-test and ANOVA, and χ² test was used for dichotomous variables. Statistical associations between two variables were assessed using simple linear-regression analyses. To account for the inflation of the experiment-wise type I error due to multiple testing, we adjusted α level downward according to the Dunn-Sidak and the Hochberg procedures (which yielded identical results). To identify independent determinants of CD34+ cell numbers, a multiple linear-regression analysis was performed with all variables listed in Supplementary Material, Table 4S entered at the same step with no step-wise selection; the predefined tolerance level to enter the equation was 0.0001. Receiver operator characteristic (ROC) curve was employed to derive sensitivity and specificity of CD34+ cell count in detecting high CV risk; the cut-off
Figure 1  Representative cytograms used for the quantitative enumeration of circulating progenitor cells according to six different phenotypes. Peripheral blood progenitor cells belong to the mononuclear cell fraction, which can be identified using a morphological gate, for their low side scatter (A). Then, we gated CD34\(^\text{+}\) (C) and CD133\(^\text{+}\) (E) cells in the mononuclear cell fraction, according to the respective isotype controls (B and D, respectively). At the intersection of the CD34 and CD133 gates, we identified CD34\(^\text{+}\)CD133\(^\text{+}\) cells in the upper-right quadrant (F). We identified in separate analyses total KDR\(^\text{+}\) cells (H) as cells with high KDR expression with respect to control (G) and low side scatter to exclude granulocytes. Gated CD34\(^\text{+}\) and CD133\(^\text{+}\) cells were examined for the dual expression of KDR (I and J, respectively) to derive the count of CD34\(^\text{+}\)KDR\(^\text{+}\) and CD133\(^\text{+}\)KDR\(^\text{+}\) cells. Triple positive cells were identified in the CD34 gate as cells positive for KDR and CD133 (K).
points have been obtained maximizing the sum of sensitivity and specificity and the 95% bias-corrected and accelerated confidence intervals were calculated using a non-parametric bootstrap resampling procedure on the basis of 2000 replicates.\textsuperscript{19} Difference between two ROC curves was assessed by the random permutation test suggested by Venkatraman and Begg.\textsuperscript{20} The independent correlation between high CV risk and risk factors or cell counts was studied using a multiple logistic regression with all variables listed in Table 1 entered as a single block at the same step. Assumption for multiple regressions was normal distribution of the response variable and independency of explanatory variables. Normality for multiple regressions was normal distribution of the response variable and independency of explanatory variables. Normality for multiple regressions was normal distribution of the response variable and independency of explanatory variables.

### Results

#### Patient characteristics and progenitor cell counts

Patient characteristics are resumed in Table 2. On average, the study sample was representative of an intermediate-to-high risk population (mean age 56.4 ± 14.9), as the mean predicted 10-year CV risk was 18% and a mean of 3.3 conventional risk factors were detected for each subject. A relatively high mean risk depended upon a study protocol allowing inclusion of patients along the entire risk range; this was decided in order to achieve more significant correlations with progenitor cell counts than if the risk range had been narrow and with a low average.

CV progenitor cells are considered to be characterized by the expression of immature markers, such as CD34 and CD133, and endothelial markers, such as KDR. With the use of these surface markers, six cell subpopulations with different antigenic profiles can be identified: CD34\textsuperscript{+}CD133\textsuperscript{−}, CD34\textsuperscript{−}CD133\textsuperscript{+}, CD34\textsuperscript{+}KDR\textsuperscript{−}, CD133\textsuperscript{−}KDR\textsuperscript{+}, and CD34\textsuperscript{−}CD133\textsuperscript{−}KDR\textsuperscript{−}. The mean quantity of CD34\textsuperscript{+} cells was 0.038 ± 0.02% of circulating leukocytes (corresponding to a range of about 250–5000 cells/mL); on average, 19.5% of CD34\textsuperscript{+} cells were expressed KDR, and as determined in a subset of 136 subjects, 41.8% of CD34\textsuperscript{+} cells also expressed KDR, and as determined in a subset of 136 subjects, 41.8% of CD34\textsuperscript{+} cells also expressed CD133, whereas 70% of CD133\textsuperscript{+} cells were CD34\textsuperscript{−}.

First, we performed a cross-analysis to determine the reciprocal relationships between progenitor cell counts obtained using different antigenic combinations, which revealed a very close linear correlation between CD34\textsuperscript{+} and CD133\textsuperscript{−} cells (r = 0.92); the presence of KDR was more restrictive, as CD34\textsuperscript{+}KDR\textsuperscript{−}, CD133\textsuperscript{−}KDR\textsuperscript{+}, and CD34\textsuperscript{−}CD133\textsuperscript{−}KDR\textsuperscript{−} cells were more weakly correlated with CD34\textsuperscript{+}, CD133\textsuperscript{−}, and CD34\textsuperscript{−}CD133\textsuperscript{−} cells, respectively. However, the numbers of KDR\textsuperscript{−} cells, which cannot be considered progenitors because of the lack of immaturity antigens, significantly correlated with progenitor cell counts (Supplementary Material, Table 2S).

Then, we obtained the coefficients of correlation between each progenitor cell’s antigenic profile with CV parameters and observed that in all cases, CD34\textsuperscript{+} cells had a closer correlation (higher Pearson’s r coefficient with lower P-value) than the other cell subtypes even when the statistical analysis was restricted to the 136 patients in which the triple marking with CD34, CD133, and KDR was performed (Figure 2; Supplementary Material, Table 3S). Moreover, only CD34\textsuperscript{+} cell count was significantly reduced in at-risk vs. healthy subjects and in patients with established CVD vs. at-risk subjects (Figure 1).

These results allowed us to focus on CD34\textsuperscript{+} cells for further analyses.

#### Relationships between CD34\textsuperscript{+} progenitor cells, risk factors, and cumulative indexes of risk

The levels of CD34\textsuperscript{+} cells in the presence or absence of classical risk factors are presented in Figure 3: diabetes mellitus, obesity, hypertension, hyperlipidaemia, and age over 50 were characterized by a significant CD34\textsuperscript{+} cell reduction. Consistently, significant CD34\textsuperscript{+} cell decrease were also documented in patients with CVD compared with those without.

Linear correlations between CD34\textsuperscript{+} cells and age (r = −0.40), blood glucose (r = 0.34), systolic blood pressure (r = −0.33), LDL (r = −0.22), HDL (r = 0.23), triglycerides (r = −0.34), and waist circumference (r = −0.23) (Figure 4) were significant after adjustment for α-inflation, whereas correlations between CD34\textsuperscript{+} cells and total cholesterol and BMI and diastolic blood pressure were not significant. In a multiple-regression analysis, age, total and LDL cholesterol, and triglyceride concentrations were significantly correlated with CD34\textsuperscript{+} cell count (Supplementary Material, Table 4S). Apparently, medications had no significant influence on CD34\textsuperscript{+} cells, because they were not independently associated with CD34\textsuperscript{+} cell count, when added to the analysis.

We noted that all components of the MetSyn, measured as both discrete and continuous, were associated with reduced CD34\textsuperscript{+} cells. Then, we divided patients into six groups according to the number of criteria of the MetSyn they fulfilled (0–5) and observed that increasing numbers of criteria was linked to decreasing levels of circulating CD34\textsuperscript{+} cells. To explore whether clustering metabolic components reduced CD34\textsuperscript{+} cells in an additional or synergistic way, we plotted the observed mean CD34\textsuperscript{+} cell levels at each number of components with the predicted CD34\textsuperscript{−} cell reduction, which was calculated using an additional model. Such analysis showed that when three or more components were

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### Table 1: Multiple logistic-regression analysis to detect independent predictors of high CV risk (10-year Framingham risk >20%)

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<th>Parameter</th>
<th>β coefficient</th>
<th>P-value</th>
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<td>CD34\textsuperscript{+} cells</td>
<td>−0.100</td>
<td>0.04</td>
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<tr>
<td>CD34\textsuperscript{+}KDR\textsuperscript{−} cells</td>
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<tr>
<td>Hypertension</td>
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<td>&lt;0.001</td>
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<tr>
<td>Family history</td>
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<td>0.30</td>
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<td>Hyperlipidaemia</td>
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<td>0.006</td>
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<tr>
<td>PANova (r² = 0.53)</td>
<td>−</td>
<td>&lt;0.001</td>
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CD34\textsuperscript{−}, but not CD34\textsuperscript{+}KDR\textsuperscript{−}, cells were independently associated with high risk, together with risk factors in the Framingham equation.
present, the observed CD34+ cell levels were lower than that predicted from the additive model (Figure 5).

Significant correlations were detected between CD34+ cells and surrogate estimates of cumulative CV risk, such as number of risk factors (r = −0.36), components of the MetSyn (r = −0.37), and 10-year CV risk according to the Italian (r = −0.42) and Framingham risk (r = −0.44, Figure 4; when the analyses was repeated excluding

<table>
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<th>Table 2  Characteristics of the study sample</th>
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<td>Characteristic</td>
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<td>Number of risk factors</td>
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<td>10-year Framingham risk (%)</td>
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<td>10-year Italian risk (%)</td>
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<tr>
<td>Aspirin (%)</td>
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<td>Other hypotensive drugs (%)</td>
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<td>Insulin (%)</td>
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<td>Oral antidiabetics (%)</td>
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Data expressed as mean (SD).
*Mean Framingham risk in high-risk subjects without CVD was 23.6%.
*P < 0.05.

Figure 2 Absolute Pearson’s r coefficients of correlation between levels of progenitor cells (defined by six different antigenic profiles) and CV parameters. The analysis was performed in the entire study population for CD34+ and CD34+KDR+ cells.
subjects with established CVD, for whom the Framingham algorithm is not applicable, a similar correlation was detected, with $r = -0.40$. To explore whether CD34$^+$ cell count may provide further information on CV risk beyond classical risk factors, a multiple logistic regression was performed and showed that CD34$^+$ (but not CD34$^+$KDR$^+$) cell count was an independent predictor of high ($>20\%$) 10-year risk, together with parameters in the Framingham equation (Table 1).

**Discussion**

In this work, we report relevant correlations between the levels of circulating progenitor cells and CV parameters in a wide-risk sample and show for the first time that CD34 identifies the progenitor cell population most closely linked to CV risk.

Experimental studies indicate a relevant contribution of bone marrow cells in re-endothelization at sites of endothelial injury and in neovascularization at sites of ischaemia. Therefore, a decrease in the endogenous pool of progenitor cells may accelerate the course of CVD and may identify an elevated CV risk.

A consensus on the methods for identification of CV progenitor cells is still not available: despite extensive functional characterization, there is no agreement on which surface antigens are best suited to enumerate these circulating cells. It is a general opinion that triple marking with CD34, CD133, and KDR is the most stringent and rigorous criterion for flow cytometry. CD34 is an adhesion molecule expressed on haematopoietic stem cells, vascular progenitors, and certain microvascular endothelium. CD133 is a surface antigen with unknown function that identifies more immature progenitors and has been proposed as the most appropriate marker for EPCs because it is not expressed on mature endothelium. CD34$^+$KDR$^+$ cells were more weakly correlated with CV risk than total CD34$^+$ cells. Recent advances in stem cell research may offer an explanation for this unexpected result. All cellular components of the CV system, including endothelial, smooth muscle, and myocardial cells, have their CD34$^+$ precursors in peripheral blood that may or may not express KDR. The various risk factors may impact with different selectivity on one or more of those committed progenitors. Therefore, despite debates and major efforts for precise functional characterization of oriented progenitor cells, it seems that a more generic antigenic characterization is suited to seize the global CV risk. This approach may lead to other advantages because single-antigen analysis brings lower costs, takes less time, and implies a lower analytical error than two- or three-colour flow cytometry. Despite the use of direct immunofluorescence, acquisition of a large number of events and proper control for non-specific binding, we found a better performance in single than double or triple antigen analyses. This may explain in part why CD34$^+$ cell count was more closely linked to CV risk than double- or triple positive cells, but does not explain a weaker correlation for CD133, which has been previously considered as more appropriate in identifying progenitor cells. However, almost all antigenic combinations showed a similar trend in reduction with respect to risk profile and/or disease status (Supplementary Material, Table 3S and Figure S1). Unfortunately, we have no
data on CD14 expression, which may provide additional information to interpret reduction of CD34\(^+\) cells.

In recent years, many biomarkers of CV risk have been discovered. However, in most cases, including C-reactive protein, the correlation with cumulative indexes of risk was lower than what we report for CD34\(^+\) cells.\(^{26}\) CD34\(^+\) cells and CD34\(^+\)KDR\(^+\) cells (to a lower extent) were also correlated with age, in agreement with previous data showing age-related reduction of CD34\(^+\)KDR\(^+\) cells in CVD patients,\(^{27}\) and impairment of cultured EPCs in parallel with endothelial dysfunction.\(^{28}\) Even if CV risk increases with age, the correlation of CD34\(^+\) cells with predicted 10-year risk was independent from age.

It has been criticized that the use of flow cytometry to count progenitor cells entails an excessive abstraction, whereas culture methods allow cell isolation, enumeration, and functional assessment.\(^{5}\) However, cultures of progenitors from peripheral blood monocytes appear spurious and time dependent.\(^{14}\) Moreover, a correlation between flow cytometry count and \textit{in vitro} function of cultured progenitor cells has been reported.\(^{12}\) Therefore, the study of surface antigens, although limited, may represent a valid alternative when dealing with large population samples, being time-saving, inexpensive, precise, and reproducible.

Given the encouraging results of recent studies, progenitor cell count may be soon included among surrogate

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**Figure 4** Significant linear correlations between CD34\(^+\) cell levels and CV/metabolic parameters. When patients were divided according to the presence or absence of CVD, the correlation between CD34\(^+\) cells and the 10-year risk is very similar.
Effect of clustered metabolic factors in reducing CD34⁺ cells

The predicted and the observed values denoted a synergistic interaction for this purpose. The observed values are plotted against predicted values, calculated using an additive model (see Methods for details). Y-error bars represent standard errors. *P < 0.05 indicates a significant difference in the observed values of CD34⁺ cell counts.

Figure 5 Levels of circulating CD34⁺ cells in subjects grouped according to the number of their metabolic factors. The observed values are plotted against predicted values, calculated using an additive model (see Methods for details). Y-error bars represent standard errors. *P < 0.05 indicates a significant difference in the observed values of CD34⁺ cell counts.

Figure 6 ROC curve assessing the performance of CD34⁺ and CD34⁺KDR⁺ cell count in identifying a predicted Framingham risk >20% in patients without established CVD. Se, sensitivity; Sp, specificity; AUC, area under curve, which indicates the accuracy of the test. Cut-off values for CD34⁺ and CD34⁺KDR⁺ cell counts were 342 (95% CI 277–375) and 26 (95% CI 16–34), respectively.

markers of CV risk.⁹ We have previously reported that both CD34⁺ and CD34⁺KDR⁺ cells correlate with risk factors and are reduced in patients with diabetes and the MetSyn.⁸ Here, we extend those results to a wider sample of subjects at different risk levels, with and without CVD, showing a better performance of CD34 evaluation alone. CD34⁺ cells were negatively affected by all components of the MetSyn, and the association of different components in the same patient further reduced CD34⁺ cells. Comparison between the predicted and the observed values denoted a synergistic effect of clustered metabolic factors in reducing CD34⁺ cells. Given that progenitor cell exhaustion is now considered an independent marker of elevated CV risk, these data are in agreement with the notion that the MetSyn is not a mere association, but its components act synergistically to worsen the risk. Our work also provides one possible mechanism linking metabolic alterations to CD34⁺ cell reduction: an analysis performed in a subgroup of 125 subjects (58% of total) indicates a negative correlation between CD34⁺ cell count and the HOMA (homeostasis model assessment) value (r = −0.18, P = 0.037), a measure of insulin resistance.⁳⁰ Molecular mechanisms underlying progenitor cell reduction in the setting of hyperlipidaemia³¹ and hyperglycaemia³² have been described, but it is not known whether hyperinsulinaemia itself exerts detrimental effects. EPC dysregulation has also been associated with impaired nitric oxide bioavailability,³³ another common feature of MetSyn.

Some drugs which have pleiotropic CV actions, including statins, ACE-inhibitors, and glitazones, have been shown to increase circulating CD34⁺KDR⁺ cells and to stimulate cultured EPCs,⁸ but we have found no effect with such medications. We hypothesize that this apparent contrast depends upon the fact that previous small short-term trials may have detected a transient phenomenon, whereas in our cross-sectional study, favourable effects of medications were masked by the negative impact of CV risk on progenitor cell levels.

The percentage of cumulative risk variance which could be explained by CD34⁺ cell count (r² = 19.3%) was higher than that of BMI (15%), diastolic blood pressure (13%), and total cholesterol (4%). CD34⁺ cell count was also an independent predictor of high CV risk, suggesting that it may provide additional information beyond the evaluation of classical risk factors. Clinical use of CD34⁺ cell count in risk assessment will need definition of a threshold. ROC curve analysis restricted to patients without established CVD, for whom the Framingham algorithm is applicable, revealed that a CD34⁺ cell count lower than 342 × 10⁶ cells has a 92% sensitivity and 70% specificity in detecting a 10-year CV risk >20% (odds ratio 2.3.6) (Figure 6). These results were similar to those obtained in a follow-up study performed to assess the ability of CD34⁺KDR⁺ cells to detect a future CV event,¹² although in our population, CD34⁺KDR⁺ cell count was less able to identify high risk. Specificity of surrogate markers, including C-reactive protein, is generally low, being affected by factors not directly linked to CV risk.³⁴

In conclusion, our study indicates that the amount of circulating CD34⁺ cells is negatively correlated with CV risk and is synergistically affected by increasing components of the MetSyn. Remarkably, single-antigen analysis with CD34 offered a better performance than double- or triple staining in detecting CV risk. These results may have important clinical implications, as enumeration of circulating progenitor cells is going to be used as a surrogate marker of risk: extensive study of surface antigen expression may be not necessary for this purpose.

Supplementary material

Supplementary material is available at European Heart Journal online.

Conflict of interest: none declared.
References


