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Circulating CD31+ Leukocyte Frequency is Associated with Cardiovascular Risk Factors

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Abstract

Objectives—CD31 identifies a heterogeneous population of cells in the blood, consisting of mature leukocytes and platelets, as well as smaller numbers of endothelial and progenitor cells. Because unfractionated CD31+ blood cells have demonstrated angiogenic properties *in vivo*, we hypothesized that circulating CD31+ cells would be related to the presence of cardiovascular risk factors in humans.

Methods and Results—We studied 1,487 participants, free of cardiovascular disease, from the Framingham Offspring Study. Using anti-human CD31 and CD45 antibodies, distinct CD31+/CD45+ leukocyte populations were enumerated in blood samples by FACS analysis. We used linear regression analyses to investigate the relation of each cell phenotype with cardiovascular

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risk factors. We identified 3 distinct leukocyte populations: CD31⁻, CD31^{dim}, and CD31^{bright} cells. Using forward/side scatter analyses, CD31⁻ and CD31^{dim} cells mapped to lymphoid gates while CD31^{bright} cells were monocytoïd. In multivariable analyses, higher frequency of CD31^{bright} cells was associated with older age, male sex, and CRP (all $P < 0.001$). In contrast, CD31^{dim} was inversely associated with age, male sex, CRP, and smoking (all $P < 0.01$). Framingham Risk Score was positively associated with CD31^{bright} frequency ($P = 0.002$), and negatively associated with CD31^{dim} frequency ($P = 0.020$).

Conclusions—CD31⁺ staining identifies 2 major leukocyte populations, CD31^{bright} and CD31^{dim}, which demonstrated significant and opposite associations with cardiovascular risk in humans. Further research is needed to define the biological and potential therapeutic roles of CD31⁺ subpopulations in vascular disease.

Keywords

epidemiology; CD31; leukocytes; endothelial cells; cardiovascular risk factors

Platelet endothelial cell adhesion molecule-1 (PECAM-1), or CD31, is a surface marker that identifies a heterogeneous population of cells in the blood that includes endothelial cells (EC), progenitor cells, platelets, and mature hematopoietic cells. With respect to ECs, CD31 has been shown to be involved in cell-to-cell contacts, providing a role in the maintenance of vascular integrity [1]. With respect to neutrophils, monocytes, and progenitor cells, CD31 is involved in mediating trans-endothelial migration, a key step in both inflammation and angiogenesis [2-4]. CD31 has been shown to promote interactions between ECs and monocytes, including the activation of antiapoptotic pathways [5].

Prior investigations suggest that the link between CD31 and cardiovascular disease is multifold. When present on ECs, CD31 may serve as a mechano-sensor to shear stress and mediate the activation of downstream pathways that eventually lead to atherosclerosis [6]. Upregulation of CD31 expression in ECs, in response to oxidized LDL, has also been shown to enhance leukocyte migration, which is important in the development of atherosclerosis [7]. Recently, circulating CD31⁺ cells have garnered interest as potential cellular therapeutic agents given their angiogenic potential. *In vitro* experiments have demonstrated that, when compared to their CD31⁻ counterparts, unselected CD31⁺ cells have increased expression of angiogenic genes and can generate a higher number of endothelial progenitor cells (EPC) [8]. In a model of hind limb ischemia, transplantation of CD31⁺ cells increased pro-angiogenic cytokines and improved capillary density and blood perfusion [9]. However, in this study, CD31⁺ cells were not fractionated into subpopulations, and therefore pro-angiogenic roles of each distinct CD31⁺ cell type was unclear. Indeed, while CD31 is often considered a marker of monocytes, T-cells that express CD31, have also been shown to play an important role in the formation and differentiation of EPCs [10].

Despite these findings, data on the association of CD31⁺ cells with cardiovascular disease in humans are scarce [11]. One small study demonstrated that circulating CD31⁺ T-cells are inversely correlated with age and Framingham risk score (FRS) [10]. In healthy volunteers, CD31⁺ cells were shown to respond to post-prandial lipemia via an increased production of reactive oxygen species [12]. Thus, variation in CD31 traits may reflect differences in cardiovascular risk. We hypothesized that individuals with a higher burden of cardiovascular risk factors would have lower quantity of circulating CD31⁺ cell quantity. Therefore, we conducted a comprehensive investigation of circulating CD31⁺ cells in a large, community-based cohort of ambulatory adults.

METHODS

Study Sample

In 1948, 5209 residents of Framingham, MA were enrolled into the Framingham Heart Study, a longitudinal cohort study aimed at identifying cardiovascular risk factors [13]. In 1971, an additional 5124 participants, offspring of the original cohort and their spouses, were enrolled in the Framingham Offspring Study [14]. Offspring Study participants received routine clinical examinations approximately every four years. On the eighth examination cycle (2005 through 2008), 1790 out of 3021 total examination attendees had CD31 measured. Of these individuals, we excluded N=15 participants due to missing covariates. After excluding individuals with prevalent cardiovascular disease (coronary heart disease, N=190; heart failure, N=20; cerebrovascular disease, N=50; and, peripheral vascular disease, N=28), a total of 1487 were included in the current analyses. All participants provided consent; study protocols were approved by the institutional review boards of the Boston University School of Medicine and Massachusetts General Hospital.

Clinical Assessment

All participants underwent a standardized medical examination and laboratory evaluation. Systolic and diastolic blood pressures were averaged between two physician-measured readings. Body mass index was calculated as weight divided by height squared (kg/m^2). After an overnight fast, blood was drawn for glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides. C-reactive protein (CRP) was measured using the immunoturbidimetric latex-enhanced high-sensitivity assay (Roche Diagnostics, Indianapolis, Ind). Diabetes mellitus was defined as either having a fasting glucose ≥ 126 mg/dL or being treated for diabetes mellitus. Use of lipid-lowering and antihypertensive agents, as well as cigarette smoking status, was self-reported.

CD31 Cell Phenotyping

For cell phenotyping, blood specimens were collected from participants between 8 and 9 a.m. after an overnight fast. As previously described, the resulting buffy coat was processed for cell phenotyping within 4 hours of specimen collection [15,16]. The following modifications were applied: buffy coat samples were diluted to 10.5 mLs with PBS (Invitrogen, Carlsbad, CA) and layered over 5 mLs Ficoll (Amersham Pharmacia Biotech, UK). Each sample was then centrifuged at 2200 rpm (800g) for 15 minutes at 10°C. Using Ficoll density-gradient centrifugation, peripheral blood cells were isolated from the buffy coat. These cells were incubated with Fc-block (Miltenyi Biotec) for 15 minutes at room temperature followed by anti-human CD31 and CD45 antibodies for 25 minutes on ice (BD-Pharmingen). Fluorescence-activated cell sorter (FACS) analysis was used to evaluate surface marker expression. Specifically, a Becton-Dickinson FACSCalibur flow cytometer, equipped with high throughput 96-well sample plates and fluorochrome-matched IgG isotype controls, was used to quantify the number of positive cells. FACS analysis was performed using FlowJo software (Tree Star Inc., Ashland, OR) by the same blinded investigator (YG). Red blood cells, platelets, and cell debris were excluded from analysis via forward and side scatter analyses. Three distinct leukocyte populations were analyzed as described below. Each cell type (CD31 bright, CD31 dim, CD31-) was reported as the frequency of the total number of gated events. As a separate experiment, we obtained additional control samples for further phenotyping of CD31 cells. These samples (N=3) underwent incubation with anti-human CD31, CD45, CD3 (T-cell marker), CD19 (B-cell marker), and CD14 (monocyte marker) antibodies and were analyzed using a Becton-Dickinson LSRII flow cytometer.

Statistical Analyses

Due to skewed distribution, triglycerides and CRP were natural log-transformed prior to analyses. We analyzed each CD31 phenotype separately in relation with the following covariates using age- and sex-adjusted linear regression models: systolic blood pressure (SBP), diastolic blood pressure (DBP), hypertension (SBP $\geq$ 140 mm Hg or DBP $\geq$ 90 mm Hg or taking anti-hypertensive medications), body mass index (BMI), waist circumference, total cholesterol, HDL cholesterol, log-triglycerides, diabetes mellitus, smoking status, log CRP, and use of statin medications. In the aforementioned analyses, associations with age were adjusted for sex and associations with sex were adjusted for age. Stepwise regression models then were used for each CD31 phenotype, using a threshold $P < 0.10$ for including a covariate in the model. All analyses were performed using SAS statistical software, version 9.2. A two-tailed P value of < 0.05 was considered statistically significant. We also used linear regression to examine the association of each CD31 phenotype with the Framingham Risk Score, which incorporates multiple cardiovascular risk factors as previously described [17].

RESULTS

Clinical characteristics of study participants are displayed in Table 1. The mean ($\pm$ standard deviation) age of the study sample was 65 ± 9 years, and 44% of participants were men. Overall characteristics of the study sample were similar to individuals from the larger Framingham cohort who did not undergo CD31 phenotyping (Supplemental Table). Flow cytometric analysis of CD31 and CD45 staining of human peripheral blood generated 3 distinct leukocyte populations: CD31 bright, CD31 dim, and CD31- cells (Figure 1B). To determine if CD31 dim and CD31 bright cells represented distinct cell types, we selected CD31 bright/CD45+ and CD31 dim/CD45+ events and backgated to forward (FSC)/side scatter (SSC) plots. CD31 dim events mapped to cells with low FSC and SSC characteristics typical of lymphoid cells (Figure 1D), whereas CD31 bright cells mapped to cells with characteristics typical of circulating monocytes (Figure 1E). In our study sample, the mean ($\pm$ standard deviation) percent of each phenotype within nucleated events were as follows: 20.9 ± 8.2 for CD31 bright, 40.2 ± 11.1 for CD31 dim, and 38.9 ± 12.7 for CD31 negative. To better characterize these cell populations, we obtained additional control samples to determine the percentage of CD14+ monocytes, CD3+ T-cells, and CD19+ B-cells within respective groups. Phenotyping of CD31 bright cells demonstrated that these almost uniformly express CD14 and were therefore monocytes, whereas CD31 dim and CD31- cells expressed a mixture of CD3 and CD19 lymphocytes (Supplemental Figure 1). In age- and sex-adjusted analyses (Table 2), CD31 bright frequency was inversely associated with total/HDL cholesterol, log triglycerides, and positively associated with age, male sex, HDL cholesterol and log CRP. In stepwise multivariable-adjusted analyses, CD31 bright remained significantly inversely associated with female sex ($\beta -4.17$; $P < 0.0001$) and total/HDL cholesterol ($\beta -0.68$ per 1-SD increment in total/HDL ratio; $P = 0.005$), and positively associated with age ($\beta 1.24$ per 1-SD; $P < 0.0001$) and log CRP ($\beta 1.12$ per 1-SD; $P < 0.0001$). After multivariable adjustment, CD31 bright was also inversely associated with BMI ($\beta -0.57$ per 1-SD increment; $P = 0.02$).

As shown in Table 3, the frequency of CD31 dim cells was inversely associated with age, male sex, log CRP, and cigarette smoking. In multivariable analyses, CD31 dim remained inversely associated with age ($\beta -1.88$ per 1-SD increment in age; $P < 0.0001$), male sex ($\beta -6.79$; $P < 0.0001$), cigarette smoking ($\beta -3.56$; $P = 0.0007$), and log CRP ($\beta -0.87$ per 1-SD; $P = 0.002$).

Overall, individuals with high CD31 bright frequency had a higher cardiovascular risk burden as represented by FRS, whereas those with high CD31 dim frequency had lower FRS

(Figure 2). Mean CD31 bright quantity was increased by 0.66 ($P=0.002$) per 1-SD increment in FRS, whereas CD31 dim cell quantity was decreased by -0.69 ($P=0.020$) per 1-SD increment in the FRS. There was no relationship between FRS and CD31- frequency. The relationship to FRS in both CD31 bright and CD31 dim groups was largely driven by age, as evidenced by lack of a significant association with the risk score not including age ($P=0.21$ and $P=0.57$, respectively).

DISCUSSION

In this study, we report the clinical correlates of circulating CD31 cells in a community-based sample of predominantly healthy men and women. Prior studies of CD31 cell phenotypes in humans have been limited to small, referral samples. To our knowledge, the present study is the first to assess the relationship of discrete CD31 cell phenotypes with cardiovascular risk factors, using a well-defined community cohort without prevalent cardiovascular disease. Our principal findings are three-fold. First, we observed that circulating CD31+ leukocytes are a heterogeneous population. CD31 bright cells were mainly composed of CD14+ monocytes whereas CD31 dim cells were composed of a mixture of T and B-cell lymphocytes (CD3 or CD19 positive respectively). Second, the CD31 bright cells were positively associated with key cardiovascular risk factors such as age, male sex, and CRP, in addition to overall FRS. Third, the CD31 dim phenotype demonstrated distinctly opposite associations with cardiovascular risk factors, including an overall negative association with FRS.

An accumulating body of experimental evidence has demonstrated associations of CD31 cell phenotypes with vascular health and disease. Early studies using unselected bone-marrow derived or peripheral blood mononuclear cells showed enhanced angiogenesis in both cardiac and peripheral limb ischemia models [18,19]. In an animal model of ischemia, Kawamoto et al. induced chronic myocardial ischemia via the placement of a left circumflex artery constrictor. Subsequent direct intramyocardial injection of CD31+, but not CD31-, mononuclear cells was shown to decrease ischemic area and enhance capillary density [20]. More recently, work by Kim et al. showed that in a heterogeneous population of circulating CD31+ cells, of which about 40% express the monocyte marker CD14, angiogenic gene expression was increased. Injection of unfractionated CD31+ cells in a hind limb ischemia model resulted in improved perfusion compared to both CD31- or total mononuclear cells [8,9]. Our data, however, demonstrate the importance of analyzing distinct CD31 subpopulations and their potential roles in vascular biology. Indeed it is possible that specific angiogenic activities reside in distinct CD31+ cell types rather than being a property of CD31+ cells in general. Further functional studies of these various groups, then, are needed.

Our study demonstrated a positive correlation between circulating CD31+ monocytes, cardiovascular risk factors (particularly age and CRP) and FRS. Early animal studies suggested a link between monocyte accumulation and blood vessel proliferation [21]. Subsequent in vitro analysis showed that EPCs which secrete proangiogenic growth factors such as vascular endothelial growth factor, hepatocyte growth factor, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor, almost ubiquitously expressed the monocyte marker CD14 [22,23]. Recent animal studies by Kim et al. supports CD31+ monocytes as key players in angiogenesis. The authors showed that the cells contained in the walls of newly formed blood vessels in acute, subacute and chronic wounds express both CD31 and F4/80, a macrophage marker [24]. In human studies, CD31 has been shown to be expressed on almost all monocytes [25,26]. Amongst the “pro-inflammatory” monocyte subset, however, CD31 expression tends to be even more ubiquitous [27]. A recent study from the Malmö Diet and Cancer cohort suggest that

circulating levels of “pro-inflammatory” monocytes directly correlate with future cardiovascular risk [28]. One potential explanation for our findings could be that circulating CD31+ monocytes reflect a compensatory response to cumulative risk exposure. Monocytes, for example, are heavily mobilized and recruited following myocardial ischemia, and play an important role in infarct healing [29]. Interestingly, the CD31 dim phenotype, composed of heterogeneous lymphocytes, displayed an inverse association with cardiovascular risk factors such as age, male sex and CRP. Prior studies have indicated that approximately 50% of circulating lymphoid cells express CD31, at a lower intensity relative to monocytes, consistent with our current findings [30]. The CD31 dim fraction, composed of both T and B cells, likely includes a subpopulation of T-cells lymphocytes, termed “angiogenic T-cells,” shown to play a role in atherosclerosis and angiogenesis, in both human and animal models. Hur et al. have shown that in mice, injection of CD3+/CD31+ cells into a hind limb ischemia model improved capillary density and limb perfusion compared to culture media or CD3+/CD31- cells [10]. Furthermore, in a small cohort of 58 participants, the authors also demonstrated that CD31+ T cells were inversely correlated with age and FRS. There is increasing evidence that subpopulations of leukocytes may differentially predict cardiovascular disease. In the LURIC study, over 3000 patients scheduled for coronary angiography underwent phenotyping of their white blood count [31]. In this high risk cohort, a predictive model adjusting for traditional cardiovascular risk factors and excluding active infections demonstrated a positive correlation between cardiovascular mortality and increasing neutrophil/lymphocyte, as well as monocyte/lymphocyte ratio. Increasing counts of monocytes and decreasing count of lymphocytes individually suggested a trend, but did not reach statistical significance, for the outcome. In our study, CD31- fraction, while also containing T- and B-lymphocytes, did not display any association with cardiovascular risk factors, suggesting that amongst lymphocytes the expression of CD31 may mark populations with relevance to vascular biology.

Our results also raise important questions relevant to the field of cell based research for therapeutic angiogenesis. An important advantage of peripherally derived CD31+ cells over other cell lines such as stem/progenitor cells or EPCs is that they are readily available and easily manipulated without the need for any mobilizing agents. For instance, in peripheral blood, CD34+/KDR+ EPCs represent fewer than 0.1% of circulating mononuclear cells [32,33], whereas up to 30% are CD31+ [9]. The results of our study demonstrate that a better understanding of the relative functional contribution of distinct cell phenotypes expressing CD31 to vascular biology in humans is needed.

Several limitations of our study merit consideration. The nature of our cross-sectional analyses does not let us determine whether the trend observed in CD31+ (bright and dim) cells is a marker of or directly contributes to cumulative cardiovascular risk exposure. Our interpretation of the CD31 dim cell data is also limited by its heterogeneity and lack of further analyses restricted to T or B-cell lymphocytes subsets. It should also be noted that we analyzed cells after enrichment for mononuclear cells, which excludes neutrophils from the above analyses. Finally, our analyses were performed in a community-based sample comprised of predominantly middle-aged to older adults of mainly European ancestry; thus, the generalizability of our findings to other populations is unknown.

In summary, we have shown that circulating CD31+ mononuclear cells contain at least two major populations with differing forward and side scatter characteristics. CD31 bright cells were composed of monocytes while CD31 dim cells were composed of lymphocytes. Interestingly, each were oppositely associated with a subset of traditional cardiovascular risk factors. Our results suggest that further investigation into the different phenotypes of cells expressing CD31+ is needed. Such research could facilitate the development of interventions for vascular and coronary ischemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): a novel vascular cell-cell adhesion molecule. *J Cell Biol.* 1991; 114:1059–1068. [PubMed: 1874786]
2. Woodfin A, Voisin MB, Nourshargh S. PECAM-1: a multi-functional molecule in inflammation and vascular biology. *Arterioscler Thromb Vasc Biol.* 2007; 27:2514–2523. [PubMed: 17872453]
3. DeLisser HM, Christofidou-Solomidou M, Strieter RM, et al. Involvement of endothelial PECAM-1/CD31 in angiogenesis. *Am J Pathol.* 1997; 151:671–677. [PubMed: 9284815]
4. Kim SW, Kim H, Yoon YS. Advances in bone marrow-derived cell therapy: CD31- expressing cells as next generation cardiovascular cell therapy. *Regen Med.* 2011; 6:335–349. [PubMed: 21548739]
5. Noble KE, Wickremasinghe RG, DeCornet C, Panayiotidis P, Yong KL. Monocytes stimulate expression of the Bcl-2 family member, A1, in endothelial cells and confer protection against apoptosis. *J Immunol.* 1999; 162:1376–1383. [PubMed: 9973392]
6. Tzima E, Irani-Tehrani M, Kiosses WB, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature.* 2005; 437:426–431. [PubMed: 16163360]
7. Hashimoto K, Kataoka N, Nakamura E, Tsujioka K, Kajiya F. Oxidized LDL specifically promotes the initiation of monocyte invasion during transendothelial migration with upregulated PECAM-1 and downregulated VE-cadherin on endothelial junctions. *Atherosclerosis.* 2007; 194:e9–17. [PubMed: 17194459]
8. Kim H, Cho HJ, Kim SW, et al. CD31+ cells represent highly angiogenic and vasculogenic cells in bone marrow: novel role of nonendothelial CD31+ cells in neovascularization and their therapeutic effects on ischemic vascular disease. *Circ Res.* 2010; 107:602–614. [PubMed: 20634489]
9. Kim SW, Kim H, Cho HJ, Lee JU, Levit R, Yoon YS. Human peripheral blood-derived CD31+ cells have robust angiogenic and vasculogenic properties and are effective for treating ischemic vascular disease. *Journal of the American College of Cardiology.* 2010; 56:593–607. [PubMed: 20688215]
10. Hur J, Yang HM, Yoon CH, et al. Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. *Circulation.* 2007; 116:1671–1682. [PubMed: 17909106]
11. Lee S, Yoon YS. Revisiting cardiovascular regeneration with bone marrow-derived angiogenic and vasculogenic cells. *Br J Pharmacol.* 2012 [E-pub ahead of print], DOI: 10.1111/j.1476-5381.2012.01857.x.
12. Jenkins NT, Landers RQ, Thakkar SR, et al. Prior endurance exercise prevents postprandial lipaemia-induced increases in reactive oxygen species in circulating CD31+ cells. *J Physiol.* 2011; 589:5539–5553. [PubMed: 21930598]
13. Dawber TR, Meadors GF, Moore FE Jr. Epidemiological approaches to heart disease: the Framingham Study. *Am J Public Health Nations Health.* 1951; 41:279–281. [PubMed: 14819398]
14. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol.* 1979; 110:281–290. [PubMed: 474565]

15. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *The New England journal of medicine*. 2003; 348:593–600. [PubMed: 12584367]
16. Duda DG, Cohen KS, Scadden DT, Jain RK. A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood. *Nat Protoc*. 2007; 2:805–810. [PubMed: 17446880]
17. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998; 97:1837–1847. [PubMed: 9603539]
18. Kamihata H, Matsubara H, Nishiue T, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 2001; 104:1046–1052. [PubMed: 11524400]
19. Iba O, Matsubara H, Nozawa Y, et al. Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. *Circulation*. 2002; 106:2019–2025. [PubMed: 12370229]
20. Kawamoto A, Tkebuchava T, Yamaguchi J, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*. 2003; 107:461–468. [PubMed: 12551872]
21. Arras M, Ito WD, Scholz D, Winkler B, Schaper J, Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J Clin Invest*. 1998; 101:40–50. [PubMed: 9421464]
22. Rehman J, Li J, Orschell CM, March KL. Peripheral blood “endothelial progenitor cells” are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation*. 2003; 107:1164–1169. [PubMed: 12615796]
23. Gulati R, Jevremovic D, Peterson TE, et al. Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. *Circ Res*. 2003; 93:1023–1025. [PubMed: 14605020]
24. Kim SJ, Kim JS, Papadopoulos J, et al. Circulating monocytes expressing CD31: implications for acute and chronic angiogenesis. *Am J Pathol*. 2009; 174:1972–1980. [PubMed: 19349357]
25. Schmeisser A, Garlich CD, Zhang H, et al. Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res*. 2001; 49:671–680. [PubMed: 11166280]
26. Stockinger H, Gadd SJ, Eher R, et al. Molecular characterization and functional analysis of the leukocyte surface protein CD31. *J Immunol*. 1990; 145:3889–3897. [PubMed: 1700999]
27. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*. 2003; 19:71–82. [PubMed: 12871640]
28. Berg KE, Ljungcrantz I, Andersson L, et al. Elevated CD14⁺⁺CD16⁻ monocytes predict cardiovascular events. *Circulation Cardiovascular genetics*. 2012; 5:122–131. [PubMed: 22238190]
29. Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. *Circulation*. 2010; 121:2437–2445. [PubMed: 20530020]
30. Ashman LK, Aylett GW. Expression of CD31 epitopes on human lymphocytes: CD31 monoclonal antibodies differentiate between naive (CD45RA⁺) and memory (CD45RA⁻) CD4-positive T cells. *Tissue Antigens*. 1991; 38:208–212. [PubMed: 1723542]
31. o Hartaigh B, Bosch JA, Thomas GN, et al. Which leukocyte subsets predict cardiovascular mortality? From the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study. *Atherosclerosis*. 2012; 224:161–169. [PubMed: 22809446]
32. Murphy C, Kanaganayagam GS, Jiang B, et al. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arterioscler Thromb Vasc Biol*. 2007; 27:936–942. [PubMed: 17255539]
33. Schmidt-Lucke C, Rossig L, Fichtlscherer S, et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation*. 2005; 111:2981–2987. [PubMed: 15927972]

Highlights

- We studied participants free of cardiovascular disease from the Framingham Offspring Study
- Using anti- CD31 and CD45 antibodies, we identified 3 distinct leukocyte populations: CD31⁻, CD31^{dim}, and CD31^{bright} cells.
- Using scatter analyses, CD31⁻ and CD31^{dim} cells mapped to lymphoid gates while CD31^{bright} cells mapped to monocytoïd gates
- Higher CD31^{bright} cell frequency was positively correlated with select traditional cardiovascular risk factors
- Higher CD31^{dim} cell frequency was inversely correlated with these risk factors

Figure 1. Expression of CD31 in peripheral circulating leukocytes

Panel A shows forward (FSC)/side scatter (SSC) plots of all nucleated events, with locations for lymphocytes, monocytes and neutrophils. Panel B represents CD31/CD45 plot from the nucleated gate, with the 3 different identified cell types. Panels C-E show CD31-, CD31 dim, and CD31 bright events, respectively, backgated to forward /side scatter plots.

Figure 2. Mean (and standard error) Framingham risk score by tertiles of CD31 bright (Panel A) and CD31 dim (Panel B) cell phenotypes. P values shown are for one-way analysis of variance.

Table 1

Sample Characteristics

Clinical Characteristic	Total Sample N=1,487
Age, years	65 ± 9
Male, %	44
Body mass index, kg/m ²	28.2 ± 5.3
Systolic blood pressure, mm Hg	128 ± 17
Diastolic blood pressure, mm Hg	74 ± 10
Hypertension	55
Diabetes	11
Total cholesterol, mg/dL	190 ± 35
HDL cholesterol, mg/dL	59 ± 18
Total/HDL cholesterol ratio	3.45 ± 1.01
Triglycerides, mg/dL *	99 (73, 138)
Current smoker, %	8
C-reactive protein, mg/L *	1.47 (0.77, 3.21)
Framingham risk score	7.5 ± 3.7

Values are shown as means (± standard deviation) or percentages.

* Values for triglycerides and C-reactive protein are shown as medians (25th, 75th percentiles).

Table 2

Clinical Correlates of CD31 bright

Clinical Covariate	Age- and Sex-Adjusted		Multivariable-Adjusted	
	Regression Coefficient* (standard error)	P value	Regression Coefficient* (standard error)	P value
Age, years	1.27 (0.21)	<0.0001	1.24 (0.22)	<0.0001
Male sex	3.40 (0.42)	<0.0001	4.17 (0.47)	<0.0001
BMI, kg/m ²	−0.32 (0.21)	0.13	−0.57 (0.24)	0.019
Waist circumference, cm	−0.17 (0.22)	0.43	—	
SBP, mmHg	−0.12 (0.22)	0.59	—	
DBP, mmHg	0.04 (0.22)	0.85	—	
Hypertension	0.26 (0.44)	0.55	—	
Diabetes	−0.20 (0.68)	0.77	—	
Total/HDL cholesterol	−0.47 (0.21)	0.027	−0.68 (0.24)	0.005
Total cholesterol, mg/dL	−0.03 (0.23)	0.89	—	
HDL cholesterol, mg/dL	0.58 (0.22)	0.009	—	
Log triglycerides, mg/dL	−0.56 (0.21)	0.008	—	
Current smoker	1.39 (0.78)	0.075	—	
Log CRP, mg/L	0.69 (0.21)	0.0009	1.12 (0.23)	<0.0001
Statin treatment	−0.70 (0.79)	0.37	—	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; CRP, c-reactive protein.

* Regression coefficients represent change in CD31 bright cells (dependent variable) per 1-SD change in continuous clinical covariates or presence (versus absence) of binary clinical covariates.

Table 3

Clinical Correlates of CD31 dim

Clinical Covariate	Age- and Sex-Adjusted		Multivariable-Adjusted	
	Regression Coefficient* (standard error)	P value	Regression Coefficient* (standard error)	P value
Age, years	−1.82 (0.28)	<0.0001	−1.88 (0.29)	<0.0001
Male sex	−6.57 (0.55)	<0.0001	−6.79 (0.56)	<0.0001
BMI, kg/m ²	−0.31 (0.28)	0.27	—	
Waist circumference, cm	−0.35 (0.28)	0.22	—	
SBP, mmHg	0.22 (0.29)	0.46	—	
DBP, mmHg	0.41 (0.29)	0.16	—	
Hypertension	0.39 (0.58)	0.50	—	
Diabetes	−0.03 (0.89)	0.97	—	
Total/HDL cholesterol	0.04 (0.28)	0.89	—	
Total cholesterol, mg/dL	−0.06 (0.30)	0.84	—	
HDL cholesterol, mg/dL	−0.19 (0.30)	0.52	—	
Log triglycerides, mg/dL	0.13 (0.28)	0.65	—	
Current smoker	−3.88 (1.02)	0.0002	−3.56 (1.05)	0.0007
Log CRP, mg/L	−0.95 (0.28)	0.0006	−0.87 (0.28)	0.002
Statin treatment	1.92 (1.03)	0.063	1.95 (1.02)	0.06

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; CRP, c-reactive protein.

* Regression coefficients represent change in CD31 bright cells (dependent variable) per 1-SD change in continuous clinical covariates or presence (versus absence) of binary clinical covariates.