

Sex Differences in Circulating Progenitor Cells

Matthew L. Topel, MD, MSc; Salim S. Hayek, MD; Yi-An Ko, PhD; Pratik B. Sandesara, MD; Ayman Samman Tahhan, MD; Iraj Hesarioeh, MD; Ernestine Mahar, BS; Greg S. Martin, MD, MSc; Edmund K. Waller, MD, PhD; Arshed A. Quyyumi, MD

Background—Lower levels of circulating progenitor cells (PCs) reflect impaired endogenous regenerative capacity and are associated with aging, vascular disease, and poor outcomes. Whether biologic sex and sex hormones influence PC numbers remains a subject of controversy. We sought to determine sex differences in circulating PCs in both healthy persons and patients with coronary artery disease, and to determine their association with sex hormone levels.

Methods and Results—In 642 participants (mean age 48 years, 69% women, 23% black) free from cardiovascular disease, we measured circulating PC counts as CD45^{med+} mononuclear cells coexpressing CD34 and its subsets expressing CD133, chemokine (C-X-C motif) receptor 4, and vascular endothelial growth factor receptor 2 epitopes using flow cytometry. Testosterone and estradiol levels were measured. After adjustment for age, cardiovascular risk factors, and body mass, CD34⁺ (β =−23%, P <0.001), CD34⁺/CD133⁺ (β =−20%, P =0.001), CD34⁺/chemokine (C-X-C motif) receptor 4–positive (β =−24%, P <0.001), and CD34⁺/chemokine (C-X-C motif) receptor 4–positive/CD133⁺ (β =−21%, P =0.001) PC counts, but not vascular endothelial growth factor receptor 2–positive PC counts were lower in women compared with men. Estradiol levels positively correlated with hematopoietic, but not vascular endothelial growth factor receptor 2–positive PC counts in women (P <0.05). Testosterone levels and PC counts were not correlated in men. These findings were replicated in an independent cohort with prevalent coronary artery disease.

Conclusions—Women have lower circulating hematopoietic PC levels compared with men. Estrogen levels are modestly associated with PC levels in women. Since PCs are reflective of endogenous regenerative capacity, these findings may at least partly explain the rise in adverse cardiovascular events in women with aging and menopause. (*J Am Heart Assoc.* 2017;6:e006245. DOI: 10.1161/JAHA.117.006245.)

Key Words: CD133 • CD34 • CXCR4 • estrogen • progenitor cell • vascular endothelial growth factor receptor 2

Cardiovascular disease (CVD) morbidity and mortality are lower in women of reproductive age compared with men despite similar age-adjusted risk profiles. However, after menopause, the incidence of CVD rapidly rises in women to equate the rates in men.¹ Multiple observational cohort studies have suggested that estradiol lends a protective effect on the vasculature, reducing endothelial dysfunction and atherosclerosis. However, clinical trials using hormone replacement therapy (HRT) in menopausal women have failed to show any benefit in improving cardiovascular outcomes.^{2–5}

From the Division of Cardiology, Emory University School of Medicine, Atlanta, GA (M.L.T., S.S.H., P.B.S., A.S.T., A.A.O.); Departments of Biostatistics and Bioinformatics (Y.-A.K.) and Pulmonary, Allergy, Critical Care and Sleep Medicine (G.S.M.), and Department of Hematology and Oncology, Winship Cancer Institute (I.H., E.M., E.K.W.), Emory University, Atlanta, GA.

Correspondence to: Arshed A. Quyyumi, MD, Division of Cardiology, Department of Medicine, Emory University School of Medicine, 1462 Clifton Rd. NE, Suite 507, Atlanta, GA 30322. E-mail: aquyyum@emory.edu
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To date, the reasons for these sex differences have been attributed to estrogens, but other mechanisms may also be responsible.⁶

Progenitor cells (PCs) play an important role in vascular repair and regeneration.^{7–9} Circulating PCs are primarily derived from the bone marrow mononuclear cell population and have differentiation potential for multiple cell lineages including hematopoietic and endothelial cells. They also directly participate in vascular repair through angiogenic and paracrine activity.^{7,8,10,11} Of particular interest are bone marrow-derived mononuclear cells that express a cluster of differentiation 34 (CD34) epitope. These PCs exhibit strong differentiation potential for hematopoietic and endothelial lineages, as well as nonhematopoietic, mesenchymal lineages, which do not express the CD45 epitope.^{7,10–13} Coexpression of CD34 with CD133, a 5-transmembrane antigen of primitive stem cells lost during maturation, identifies a PC-enriched population (CD34⁺/CD133⁺) with greater proliferative activity.^{14,15} Vascular endothelial growth factor receptor 2 (VEGF2R) coexpression with CD34 is a rare subpopulation of PCs (CD34⁺/VEGF2R⁺), which has greater potential for endothelial differentiation.^{16–18} Lastly, chemokine (C-X-C motif) receptor 4 (CXCR4) coexpression with CD34

Clinical Perspective

What Is New?

- Women have lower numbers of circulating progenitor cells compared with men, and estrogen levels correlate with progenitor cell numbers in women; however, testosterone levels are not associated with progenitor cells in men.

What Are the Clinical Implications?

- There is an age-related decline in circulating progenitor cells, and low levels have been associated with adverse outcomes, suggesting that progenitor cell counts represent endogenous regenerative capacity. Lower circulating levels of progenitor cells in women, compared with men, implies potential sex-based differences in regenerative capacity.

characterizes PCs (CD34⁺/CXCR4⁺) with greater capacity for tissue repair via homing of PCs to stromal-derived factor-enriched hypoxic environments.¹⁹

Despite robust data indicating that lower circulating PC counts and impaired PC activity are associated with CVD and adverse events,^{20–23} the few studies that have examined sex differences in PCs have reported conflicting findings.^{24–28} Experimental studies have demonstrated that estrogens stimulate endothelial PC activity.²⁹ We sought to characterize the sex differences in both circulating endothelial and hematopoietic PCs. We hypothesized that women would have higher numbers of all PCs compared with men, primarily attributable to the effect of estradiol, and that menopause would be associated with a reduction in PCs in women.

Material and Methods

Study Design and Patients

We recruited 467 women and 202 age-matched men without a known history of CVD from the CHDWB (Emory-Georgia Tech Center for Health Discovery and Well-Being) cohort study in Atlanta, GA. Full details of this cohort have been previously published.^{29,30} Briefly, participants were a random convenience sample of employees of Emory University and the Georgia Institute of Technology identified through the human resources department at each institution. Individuals eligible for study enrollment must have been employed for at least 2 years and covered by a university-sponsored health insurance plan. Approximately 10 000 employees were eligible based on these criteria, and every 10th employee was invited to participate. Approximately 30% of invited employees were screened, with ≈10% ultimately enrolled in the cohort. Individuals with hospitalization in the preceding year, poorly controlled or acute medical conditions, or active pregnancy were excluded. All patients were provided with and gave

written informed consent at the time of enrollment, and the study was approved by the Emory University institutional review board.^{30,31}

Demographic characteristics, medical and reproductive history, medication use, and behavioral habits were documented. Blood samples for cardiovascular risk factors, circulating PCs, and sex hormones were collected. Anthropometric data such as blood pressure (BP) and weight were measured.

To confirm our findings, we analyzed participants enrolled in the Emory Cardiovascular Biobank.^{22,32} Briefly, patients undergoing cardiac catheterization for the evaluation of known or suspected coronary artery disease (CAD) were prospectively enrolled at 3 sites within the Emory Healthcare network in Atlanta, GA, between 2003 and 2009. Exclusion criteria for participation in the study included age younger than 20 years or older than 90 years, congenital heart disease, severe anemia or recent transfusion of blood products, active infection (including myocarditis), heart transplant, or other conditions requiring immunosuppressive agents (including cancer). Demographics, medical and personal history, medication use, and behavioral characteristics were collected from questionnaires and supported by medical record review and physician evaluation. All participants were provided with and gave written informed consent at the time of enrollment, and the study was approved by the Emory University institutional review board. Patients with full PC characterization were included in the study.

PC Assays

Cell populations enriched for circulating PCs were enumerated using flow cytometry as CD45^{med} cells coexpressing CD34⁺, CD133⁺, VEGF2R⁺, or CXCR4⁺ as previously described.^{22,33–38} For each sample, 300 μL of peripheral blood was incubated with the following fluorochrome-labeled monoclonal anti-human mouse antibodies in the dark for 15 minutes: FITC-CD34 (BD Biosciences), PerCP-CD45 (BD Biosciences), PE-VEGF2R (R&D system), APC-CD133 (Miltenyi), and PE-Cy7-conjugated anti-CXCR4 (EBioscience, clone 12G5). Red blood cells were removed by lysis with 1.5 mL of ammonium chloride lysing buffer after an additional 10 minutes of incubation. Lysis was stopped with 1.5 mL of staining medium (PBS with 3% heat-inactivated serum and 0.1% sodium azide). Before flow cytometry, 100 μL of AccuCheck Counting Beads (Invitrogen, category No.: PCB100) were added to act as an internal standard for direct estimation of the concentration of target cell subsets. Up to 5 million events, but at least 2 million events, were acquired from the flow cytometer and flow data were analyzed with FlowJo software (Treestar, Inc.). Absolute mononuclear cell count was calculated as the total of all lymphocytes and monocytes measured with a Coulter ACT/Diff cell counter (Beckman

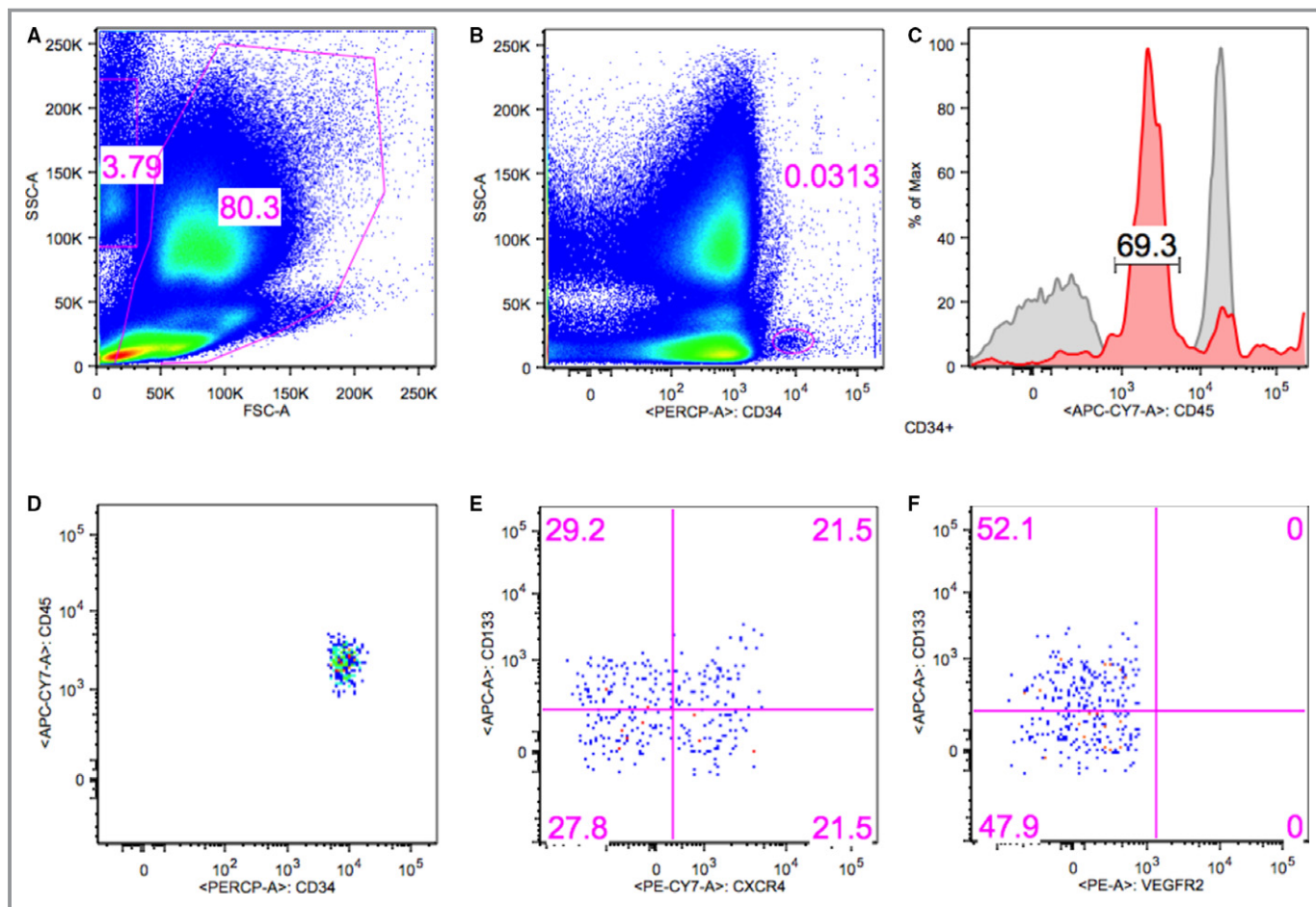


Figure 1. Flow cytometry analysis of blood progenitor cells. A, Forward scatter and side scatter gates following lyse-no wash of blood and the addition of fluorescent counting beads (left upper corner in plot). B, Gating of CD34⁺, low side scatter cells from blood leukocytes shown in (A). C, Histograms of CD45 expression in the CD34⁺ low side scatter cells from blood leukocytes shown in (B) or the CD34⁻ cells (grey histogram). D, The pattern of coexpression of CD34 and CD45^{dim} on blood progenitors shown in (C). E, The coexpression of CD133 and chemokine (C-X-C motif) receptor 4 (CXCR4) on CD34⁺CD45^{dim} blood progenitors shown in (C). F, The coexpression of CD133 and vascular endothelial growth factor receptor 2 (VEGF2R) on CD34⁺CD45^{dim} blood progenitors shown in (C). FSC-A indicates forward scatter area.

Coulter).^{33,34} PC populations are reported as cell counts per milliliter (Figure 1).

To assess repeatability, 20 samples were analyzed on 2 occasions by the same technician with the following coefficients of variation: CD34⁺ 2.9%; CD34⁺/CD133⁺ 4.8%; CD34⁺/CXCR4⁺ 6.5%; CD34⁺/VEGF2R⁺ cells 21.6%; and CD34⁺/CD133⁺/CXCR4⁺ 7.5%. There were significant correlations between the PC subtypes, with strong correlations between CD34⁺, CD34⁺/CD133⁺, and CD34⁺/CXCR4⁺ (r range 0.68–0.90, $P<0.001$), and weak correlations between CD34⁺/VEGF2R⁺ and the aforementioned PCs (r range 0.18–0.24, $P<0.001$).

Sex Hormones and Menopause Definition

The men and women had sex hormone levels assessed from random fasting blood samples. Menopause and use of HRT

were defined by patient response to a reproductive health questionnaire, age, and estradiol level.

Of the 467 women in the cohort, 15 did not have complete data to determine menopause status. An additional 12 women reported HRT use while concomitantly reporting premenopausal status, age younger than 50 years, estradiol >30 pg/mL, and no history of complete or partial hysterectomy; these patients were excluded.

Statistical Analysis

Patient characteristics were reported as means and SDs for normal continuous variables, medians and interquartile ranges for non-normal continuous variables, and counts and proportions for categorical variables. Variables were visually assessed for normality by distribution plots and Q-Q plots, as well as quantitatively using the Komogorov-Smirnov

D statistic. The overall cohort was divided by sex, and further by menopause status and HRT use, and differences between groups were assessed using *t* tests for normal continuous variables, Mann-Whitney *U* tests for non-normal continuous variables, and chi-square or Fisher exact tests for categorical variables, where appropriate. Before multivariable analyses, estradiol and PC counts for CD34⁺, CD34⁺/CD133⁺, CD34⁺/CXCR4⁺, and CD34⁺/CXCR4⁺/CD133⁺ were log-transformed (base 10) for normality. CD34⁺/VEGF2R⁺ cell counts were analyzed as a dichotomous variable using the median value (40 cells/mL) as a cutoff. We investigated the association between PCs and sex hormones using Pearson correlation coefficients. To test the association between sex and PCs, we used multivariable generalized linear models and binary logistic regression

models to adjust for the following covariates: age, sex, race, body mass index, smoking history, hypertension, diabetes mellitus, and hyperlipidemia. A 2-tailed $P \leq 0.05$ was considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute Inc).

Results

Characteristics of the Healthy Cohort

Demographic and clinical characteristics of the total cohort and of the cohort dichotomized by sex (men versus women) are shown in Table 1. Women were more likely to be black and less likely to smoke cigarettes or have dyslipidemia. They additionally had lower BP, triglyceride and fasting glucose

Table 1. Clinical Characteristics of Participants, Stratified by Sex

	All (N=642)	Men (n=202)	Women (n=440)	P Value
Age, y	47.9±10.4	48.3±10.7	47.7±10.3	0.520
Black race, No. (%)	149 (23.2)	20 (9.9)	129 (29.3)	<0.001*
Ever smoker, No. (%)	35 (5.5)	17 (8.5)	18 (4.1)	0.026*
Diabetes mellitus, No. (%)	68 (10.6)	18 (9.0)	50 (11.4)	0.358
Hypertension, No. (%)	214 (33.4)	64 (31.8)	150 (34.1)	0.575
Dyslipidemia, No. (%)	90 (14.0)	52 (25.9)	38 (8.6)	<0.001*
Systolic BP, mm Hg	120.4±16.1	122.7±14.6	119.4±16.7	0.012*
Diastolic BP, mm Hg	75.9±11.0	79.2±11.1	74.5±10.6	<0.001*
Body mass index, kg/m ²	26.5 [23.6–30.7]	26.8 [25.0–30.0]	26.2 [22.7–31.2]	0.240
Total cholesterol, mg/dL	194.2±36.1	189.1±35.1	196.5±36.4	0.016*
LDL cholesterol, mg/dL	110.6±31.7	113.5±30.0	109.2±32.3	0.108
HDL cholesterol, mg/dL	63.4±18.3	51.7±12.6	68.8±18.0	<0.001*
Triglycerides, mg/dL	86 [65–119]	100 [76–145]	80 [63–107]	<0.001*
Fasting glucose, mg/dL	87 [81–92]	88 [84–95]	86 [80–92]	<0.001*
Estradiol, pg/mL	43 [25–92]	...*
Total testosterone, ng/dL	...	456 [342–582]
Free testosterone, pg/mL	...	62 [48–78]
Bioavailable testosterone, ng/dL	...	131 [104–171]
CD34 ⁺ , cells/mL	2095 [1312–3172]	2628 [1732–3993]	1925 [1200–2768]	<0.001*
CD34 ⁺ /CD133 ⁺ , cells/mL	900 [557–1404]	1128 [699–1691]	812 [522–1295]	<0.001*
CD34 ⁺ /CXCR4 ⁺ , cells/mL	855 [515–1385]	1132 [704–1699]	753 [476–1266]	<0.001*
CD34 ⁺ /VEGF2R ⁺ , cells/mL	36 [9–98]	28 [6–95]	41 [11–99]	0.203
CD34 ⁺ /CXCR4 ⁺ /CD133 ⁺ , cells/mL	348 [205–525]	405 [275–653]	308 [190–479]	<0.001*
CD34 ⁺ /VEGF2R ⁺ /CD133 ⁺ , cells/mL	12 [0–38]	10 [0–36]	12 [0–40]	0.230
CD34 ⁺ /CXCR4 ⁺ /VEGF2R ⁺ , cells/mL	35 [10–94]	30 [7–92]	37 [11–94]	0.178

Values are expressed as number (percentage of prevalence) for categorical variables, mean±SD for normal continuous variables, and median [interquartile range] for non-normal continuous variables. BP indicates blood pressure; CXCR4, chemokine (C-X-C motif) receptor 4; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VEGF2R, vascular endothelial growth factor receptor 2.

*Significant differences between men and women.

Table 2. Clinical Characteristics of Women, Stratified by Menopause Status and HRT Use, and Age-Matched Men

	Premenopausal Women (n=264)	Age-Matched Young Men (n=123)	Menopausal Women (n=91)	Age-Matched Older Men (n=39)	Menopausal Women on HRT (n=71)	Age-Matched Older Men (n=40)
Age, y	41.5±8.0	42.5±9.2	56.0±4.4*	56.7±4.8*	57.7±5.8*	57.8±5.9*
Black race, No. (%)	89 (33.7)	16 (13.0) [†]	24 (26.4)	0 (0.0) [†]	10 (19.3)	4 (10.0)
Ever smoker, No. (%)	11 (4.2)	9 (7.3)	2 (2.2)	3 (7.9)	5 (6.1)	5 (12.5)
Diabetes mellitus, No. (%)	24 (9.1)	11 (9.0)	16 (17.6)	4 (10.3)	10 (12.1)	3 (7.5)
Hypertension, No. (%)	72 (27.3)	37 (30.3)	42 (46.1)	16 (41.0)	36 (43.4)	11 (27.5)
Dyslipidemia, No. (%)	9 (3.4)	21 (17.2) [†]	16 (17.6)	18 (46.2) [†]	12 (14.5)	13 (32.5) [†]
Systolic BP, mm Hg	116.3±15.4	120.6±14.6 [†]	123.1±15.6*	126.7±15.0	124.6±19.3*	125.0±13.0
Diastolic BP, mm Hg	73.5±10.6	77.5±11.5 [†]	76.7±10.0*	81.9±10.3 [†]	75.4±11.3	81.5±9.5 [†]
Body mass index, kg/m ²	26.0 [22.3–31.4]	26.7 [24.9–29.7]	26.4 [23.2–31.1]	27.1 [25.5–30.6]	27.2 [23.9–31.1]	26.4 [24.5–30.0]
Total cholesterol, mg/dL	189.8±33.9	190.8±36.3	209.6±37.3*	184.3±30.6 [†]	204.0±37.6*	188.6±35.7 [†]
LDL cholesterol, mg/dL	105.8±31.1	116.1±30.8 [†]	117.2±31.2*	107.3±28.3	111.7±35.0	111.9±28.7
HDL cholesterol, mg/dL	67.0±16.5	50.9±13.6 [†]	72.5±20.2*	52.2±11.0 [†]	70.5±19.1	53.4±11.0 [†]
Triglycerides, mg/dL	78.0 [60.0–100.0]	99.0 [75.0–142] [†]	88.0 [65.0–111.0]*	108.5 [79.0–154.0] [†]	87.5 [64.0–132.0]*	107.5 [79.5–148.5]
Fasting glucose, mg/dL	84.0 [79.0–89.0]	89.0 [83.0–95.0] [†]	88.0 [82.0–94.0]*	88.0 [84.0–93.0]	88.5 [82.0–95.0]*	89.0 [86.0–100.5]
Estradiol, pg/mL	68.0 [38.0–151.0]	...	24.0 [17.0–35.0]*	...	34.5.0 [23.0–57.0]*	...
Total testosterone, ng/dL	...	476 [368–596]	...	401 [323–561]	...	420 [305–547]
Free testosterone, pg/mL	...	71 [50–87]	...	59 [48–70]	...	52 [47–62]*
Bioavailable testosterone, ng/dL	...	150 [108–190]	...	88 [84–93] [†]	...	109 [93–132]*
CD34 ⁺ , cells/mL	1973 [1261–2926]	2727 [1722–4170] [†]	1945 [1173–2710]	2706 [1937–3905] [†]	1638 [1067–2566]	2350 [1474–3450] [†]
CD34 ⁺ /CD133 ⁺ , cells/mL	854 [572–1368]	1160 [722–1754] [†]	787 [488–1157]	1247 [806–1691] [†]	688 [448–966]*	960 [580–1523] [†]
CD34 ⁺ /CXCR4 ⁺ , cells/mL	772 [490–1280]	1174 [697–1727] [†]	788 [488–1248]	1192 [758–1659] [†]	668 [351–1254]	1058 [54–1556] [†]
CD34 ⁺ /VEGF2R ⁺ , cells/mL	46 [10–109]	28 [6–107]	35 [11–98]	40 [12–94]	35 [13–87]	18 [0–58]
CD34 ⁺ /CXCR4 ⁺ /CD133 ⁺ , cells/mL	316 [197–524]	430 [264–663] [†]	338 [186–471]	398 [308–685] [†]	262 [183–400]	374 [243–562] [†]
CD34 ⁺ /VEGF2R ⁺ /CD133 ⁺ , cells/mL	15 [0–40]	10 [0–37]	10 [0–42]	14 [6–40]	11 [0–31]	8 [0–27]
CD34 ⁺ /CXCR4 ⁺ /VEGF2R ⁺ , cells/mL	42 [11–97]	29 [6–95]	31 [10–97]	39 [12–94]	32 [14–85]	16 [0–68]

Values are number (percentage) for categorical variables, mean±SD for normal continuous variables, and median [interquartile range] for non-normal continuous variables. BP indicates blood pressure; CXCR4, chemokine (C-X-C motif) receptor 4; HDL, high-density lipoprotein; HRT, hormone replacement therapy; LDL, low-density lipoprotein; VEGF2R, vascular endothelial growth factor receptor 2.
 *P<0.05, for menopausal vs premenopausal women or older vs younger men.
[†]P<0.05, for women vs age-matched men.

levels, and higher total and high-density lipoprotein cholesterol compared with men (Table 1).

The cohort was further divided into subgroups for premenopausal and menopausal women, with or without the use of HRT, and age-matched men (Table 2). Similar differences between each menopause subgroup of women and their age-matched male cohorts were present—men were generally less likely to be black and more likely to have dyslipidemia. They were also more likely to have higher BPs, primarily diastolic BP, higher total and low-density lipoprotein cholesterol, lower high-density lipoprotein cholesterol, and higher triglycerides (Table 2).

Regardless of HRT use, menopausal women were older than premenopausal women and had greater levels of age-related risk factors, including BP, cholesterol, and glucose. Menopausal women taking HRT had lower estradiol levels than premenopausal women, and menopausal women not taking HRT had lower estradiol levels than either premenopausal women or menopausal women taking HRT (Table 2).

Relationship Between PCs and Sex

In unadjusted analyses, PC counts enriched for hematopoietic progenitors (CD34⁺, CD34⁺/CD133⁺, CD34⁺/CXCR4⁺, and CD34⁺/CXCR4⁺/CD133⁺) were lower in women compared with men; however, cell counts for PCs enriched for endothelial progenitors (VEGF2R⁺-expressing cell subsets) were not different between men and women (Table 1, Figure 2). This remained true for subgroups of women based on menopause status and age-matched men (Table 2).

On multivariable analysis adjusting for age, race, smoking history, body mass index, hypertension, diabetes mellitus,

dyslipidemia, and mononuclear cell count, female sex remained an independent determinant of PC counts and was associated with 23% fewer CD34⁺ cells ($P<0.001$), 20% fewer CD34⁺/CD133⁺ cells ($P=0.001$), 24% fewer CD34⁺/CXCR4⁺ cells ($P<0.001$), and 21% fewer CD34⁺/CXCR4⁺/CD133⁺ cells ($P=0.001$) compared with men. There was no association between sex and CD34⁺/VEGF2R⁺ cells ($P=0.09$).

The age-related decline in circulating PCs is shown in Figure 3. The interaction between age and sex was tested, given previously described associations between PC counts and age³³; however, age did not affect the association between sex and PC counts (Figure 3). Furthermore, tests for interaction between menopause status and PC counts were negative and did not affect the association between sex and PC counts.

Relationship Between PCs and Sex Hormones

Among women, estradiol weakly correlated with hematopoietic PC counts—CD34⁺ ($r=0.13$, $P=0.007$), CD34⁺/CD133⁺ ($r=0.11$, $P=0.027$), CD34⁺/CXCR4⁺ ($r=0.14$, $P=0.003$), and CD34⁺/CXCR4⁺/CD133⁺ ($r=0.14$, $P=0.005$)—but not the VEGF2R⁺-expressing PCs ($r=-0.01$, $P=0.98$; not shown) (Table 3). Among men, there were no significant correlations between testosterone levels and either hematopoietic or endothelial PCs (Table 3). On multivariable analysis, estradiol was not an independent determinant of either hematopoietic or endothelial PC counts in women.

Relationship Between Menopause Status in Women and PCs

There were no differences in PC counts between premenopausal and menopausal women with or without HRT use (Table 4). Further comparisons between premenopausal and menopausal women with age-matched men showed that men had higher hematopoietic PC counts regardless of female menopause status or HRT use (Table 2).

Replication of Findings in a Cohort With CAD

Characteristics of the 1728 patients from the Emory Cardiovascular Biobank are shown in Table 5. Women were less likely to be black and more likely to smoke cigarettes. In addition, women had lower body mass index and higher prevalence of CAD (Table 5). In unadjusted analyses, PC counts enriched for hematopoietic progenitors (CD34⁺, CD34⁺/CD133⁺, and CD34⁺/CXCR4⁺) were lower in women compared with men. In this cohort with CAD, cell counts for PCs enriched for endothelial progenitors (CD34⁺/VEGF2R⁺) were also significantly lower in women compared with men (Table 5).

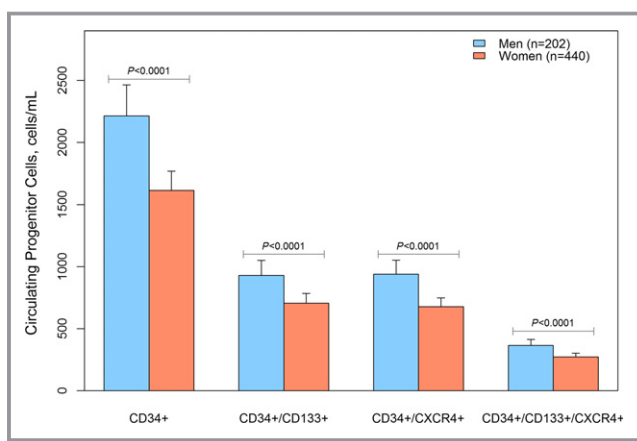


Figure 2. Sex differences in hematopoietic progenitor cells in healthy individuals. For all hematopoietic progenitor cell populations, men had higher numbers of cells after adjusting for age, race, and body mass index.

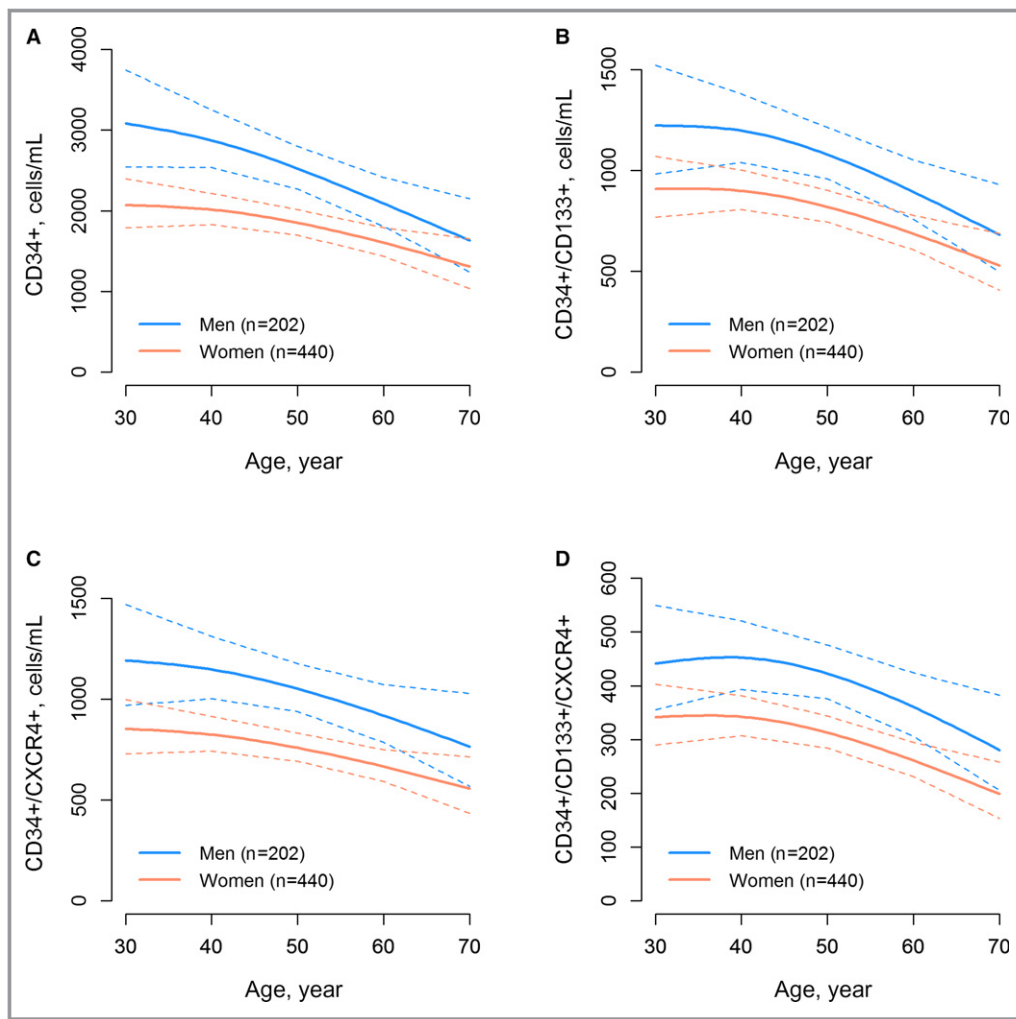


Figure 3. The effect of aging on differences between hematopoietic progenitor cells in healthy individuals. Men continue to have greater numbers of CD34⁺(A), CD34⁺/CD133⁺ (B), CD34⁺/chemokine (C-X-C motif) receptor 4 (CXCR4⁺) (C), and CD34⁺/CD133⁺/CXCR4⁺ (D) cells throughout their life course compared with women; however, these differences attenuate later in life.

After multivariable adjustment for demographics, behavioral characteristics, medical history, and mononuclear cell counts, female sex remained an independent determinant of PC counts and was associated with 15% lower CD34⁺ cells

(*P*=0.002), 12% lower CD34⁺/CD133⁺ cells (*P*=0.032), and 19% lower CD34⁺/CXCR4⁺ cells (*P*<0.001). Female sex was also associated with decreased odds of CD34⁺/VEGF2R⁺ >40 cells/mL (odds ratio, 0.611; *P*<0.001).

Table 3. Pearson Correlation Coefficients for Hematopoietic Progenitor Cells and Sex Hormones, Stratified by Sex

Variables	Women (n=440)				Men (n=202)			
	CD34 ⁺	CD34 ⁺ /CD133 ⁺	CD34 ⁺ /CXCR4 ⁺	CD34 ⁺ /CXCR4 ⁺ /CD133 ⁺	CD34 ⁺	CD34 ⁺ /CD133 ⁺	CD34 ⁺ /CXCR4 ⁺	CD34 ⁺ /CXCR4 ⁺ /CD133 ⁺
Estradiol, pg/mL	0.13*	0.11*	0.14*	0.14*				
Total testosterone, ng/dL					-0.06	-0.07	-0.04	-0.07
Free testosterone, pg/mL					0.04	0.05	0.02	0.01
Bioavailable testosterone, ng/dL					0.05	0.05	0.03	0.01

Pearson correlation coefficients between progenitor cells and sex hormones. All variables are log-transformed (base 10) for the purposes of correlation analysis. **P*<0.05.

Table 4. Circulating Progenitor Cells in Healthy Premenopausal and Menopausal Women, With and Without HRT Use

	Premenopausal Women (n=264)	Menopausal Women (n=91)	Menopausal Women on HRT (n=71)	P Value
CD34 ⁺ , cells/mL	1973 (1261–2926)	1945 (1173–2710)	1638 (1067–2566)	0.066
CD34 ⁺ /CD133 ⁺ , cells/mL	854 (572–1368)	787 (488–1157)	688 (448–966)	0.111
CD34 ⁺ /CXCR4 ⁺ , cells/mL	772 (490–1280)	788 (488–1248)	668 (351–1254)	0.120
CD34 ⁺ /VEGF2R ⁺ , cells/mL	46 (10–109)	35 (11–98)	35 (13–87)	0.266
CD34 ⁺ /CXCR4 ⁺ /CD133 ⁺ , cells/mL	316 (197–524)	338 (186–471)	262 (183–400)	0.095
CD34 ⁺ /VEGF2R ⁺ /CD133 ⁺ , cells/mL	15 (0–40)	10 (0–42)	11 (0–31)	0.366
CD34 ⁺ /CXCR4 ⁺ /VEGF2R ⁺ , cells/mL	42 (11–97)	31 (10–97)	32 (14–85)	0.395

CXCR4 indicates chemokine (C-X-C motif) receptor 4; HRT, hormone replacement therapy; VEGF2R, vascular endothelial growth factor receptor 2. Values are median (interquartile range).

Discussion

In the largest study to date of healthy individuals free of known CVD investigating the influence of sex on circulating PCs, we demonstrate that compared with men, women have lower circulating PC subsets enriched for hematopoietic progenitors. Female sex was associated with 20% to 24% fewer PCs after adjusting for common cardiovascular risk factors. Although estradiol was associated with hematopoietic PC counts, there were no differences in PCs between premenopausal and menopausal women, and age-matched men for each cohort had significantly higher PC counts than either subset of women. Lastly, these findings were replicated in a separate cohort of older individuals with prevalent CVD, and women had 12% to 19% fewer hematopoietic PCs compared with men. Thus, in two cohorts exceeding 2300 patients with and without CVD, circulating PCs enriched for hematopoietic progenitors were lower in women than in men.

The impact of sex on CVD, and particularly the lower prevalence of CVD in younger women, has focused largely on the role estrogens. More recent, the impact of estrogens on endothelial PC number and function has emerged as a possible mechanism for this finding.^{39,40} Experimental models showed a clear association between levels of estrogen and the number and function of endothelial PCs.^{41,42} Estrogens appear to upregulate expression of estrogen receptors, specifically estrogen receptor α , resulting in greater PC homing.^{43,44} Clinical studies have shown that prolonged estrogen exposure leads to increased estrogen receptor expression in both cultured and circulating human endothelial PCs.⁴⁵

Several human studies have explored the relationship between PCs and estrogens. Fadini et al²⁷ demonstrated that PC levels change throughout the menstrual cycle in parallel with estrogen levels. Lemieux et al⁴⁶ reported that estrogen influences endothelial PC number and maturation throughout the menstrual cycle. Robb et al⁴⁷ also demonstrated that the

absolute number of endothelial PCs vary over the menstrual cycle in premenopausal women, but found no differences in PC functional assays with respect to estrogens. Finally, da Silva et al²⁸ showed that low-dose estrogen therapy in menopausal women increased mobilization of endothelial PCs from the bone marrow. These findings are consistent with our observed modest correlation between estrogen levels and circulating PC counts.

While the effect of estrogen on endothelial PCs has been well documented, reports on sex differences in PC levels have been studied in small populations with less consistent findings. For example, Stauffer et al²⁵ found no differences in endothelial PCs, defined as CD34⁺/CD133⁺/VEGF2R⁺, in 29 menopausal women compared with men. Similarly, no sex-related differences in healthy young men and women were reported by Ruszkowska-Ciastek et al.⁴⁸ Four studies have found higher numbers of endothelial PCs in women compared with men. Pelliccia et al⁴⁹ found that only menopausal women without CAD had higher PCs than their age-matched male cohort. Lemieux et al⁴⁶ demonstrated that women have greater mean numbers of endothelial PCs, defined as CD133⁺/CD34⁺ and CD133⁺/VEGF2R⁺ cells, compared with men, a finding similar to that reported by Hoetzer et al²⁴ in healthy menopausal women. Finally, in 210 premenopausal and menopausal women compared with age-matched male cohorts, Fadini et al²⁷ found that premenopausal women had higher CD34⁺/KDR⁺, but not CD133⁺/KDR⁺ or CD34⁺/CD133⁺/KDR⁺, PC counts compared with age-matched men and menopausal women.

These studies were small, used different assays to study PCs, and largely concentrated on endothelial PC populations. Our study is larger, shows clear and reproducible differences (particularly in hematopoietic PCs, a population that reflects regenerative capacity), and is predictive of long-term outcomes.²² We also found that these differences persisted throughout the lifespan, even as the PC counts declined with

Table 5. Characteristics of Patients With CVD, Stratified by Sex

Variables	Men (n=1061)	Women (n=667)	P Value
Age, y	66±13	64±13	0.666
Black race, No. (%)	200 (30)	213 (20)	<0.001*
Body mass index, kg/m ²	30±7	29±6	<0.001*
Clinical characteristics, No. (%)			
Smoking history	398 (60)	751 (71)	<0.001*
Hypertension	600 (91)	949 (90)	0.799
Diabetes mellitus	279 (43)	438 (42)	0.911
Hyperlipidemia	470 (71)	789 (75)	0.078
Coronary artery disease	573 (86)	960 (91)	0.003*
Peripheral vascular disease	118 (18)	208 (20)	0.301
Heart failure	182 (28)	297 (29)	0.721
Circulating progenitor cells, cells/mL			
CD34 ⁺	1818 [1140–2796]	1586 [1003–2368]	<0.001*
CD34 ⁺ /CD133 ⁺	820 [495–1335]	745 [448–1150]	0.002*
CD34 ⁺ /CXCR4 ⁺	907 [529–1492]	738 [448–1288]	0.001*
CD34 ⁺ /VEGFR2 ⁺	44 [13–147]	30 [9–110]	<0.001*

CVD indicates cardiovascular disease; CXCR4, chemokine (C-X-C motif) receptor 4; VEGFR2, vascular endothelial growth factor receptor 2.

Values are number (percentage of prevalence) for categorical variables, mean±SD for normal continuous variables, and median [interquartile range] for non-normal continuous variables.

*Significant differences between men and women.

aging in both sexes.³³ Thus, the hypothesis that women have lower CVD burden because of higher PC levels requires reevaluation. An alternative explanation involves the role that estrogen plays in preventing PC senescence through a variety of mechanisms, including its activating effect on telomerase expression and activity.⁵⁰ We have recently reported that leukocyte telomere length is associated with decreased PC counts and that both decreased PC counts and shorter leukocyte telomere length are independently associated with worse cardiovascular outcomes.⁵¹ As women transition through menopause and estrogen exposure decreases, the deleterious effects of PC senescence, reduced telomere length, and lower absolute PC levels compared with men may explain the accelerated rate of cardiovascular outcomes in women for this advanced age group.

Study Strengths and Limitations

Strengths of our study include evaluation of PCs in two separate cohorts with and without prevalent CAD, leading to its large size. We also have uniform enumeration of PCs by the same laboratory with comprehensive investigation of both hematopoietic and endothelial-enriched CD34⁺ subpopulations. Lastly, we were able to incorporate male and female sex hormones to assess their associations with PCs in our cohort

without prevalent CAD. Limitations include its cross-sectional design, which prevents determination of casual links between sex, sex hormones, and PCs. Differences in risk factor profiles between men and women cannot be excluded as contributing to the observed differences in PCs, despite statistical adjustment through multivariable modeling. The questionnaire used to determine reproductive and menopause history did not ask for specific HRT formulations or duration of use, further limiting causal inference. In addition, both testosterone and estrogen have diurnal variation, and estrogen varies throughout the menstrual cycle⁵²; hormone samples were not drawn explicitly to address these variations.

Conclusions

We and others have shown that PC counts decrease with aging, exposure to CVD risk factors, or prevalent CVD, and low levels of PCs are associated with increased risk of CVD events.²² Because women have lower PC counts compared with men, they are likely to reach a critically low level with aging that is associated with increased risk of adverse CVD outcomes. This may explain why the risk of CVD rapidly rises in women with aging and menopausal status, and further studies examining the impact of estrogen on regenerative capacity at the time of menopause are warranted.

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Disclosures

None.

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