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RESEARCH LETTER

Fraction of Osteocalcin Endothelial Progenitor Cells and Cardiovascular Risk

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Endothelial progenitor cells (EPCs) play a role in vascular repair, and their levels might represent cardiovascular health.[1] Contrarily, osteocalcin-expressing EPCs (OC-EPCs) have previously been linked to coronary artery disease.[2,3] However, their association with cardiovascular risk factors and long-term prognosis is yet to be elucidated. Here, we aimed to investigate this aspect of the EPCs further.

Between September 2006 and October 2020, 1046 patients visiting our Cardiology department were enrolled in the prospective research database to enumerate EPCs. The study has been approved by our institutional review board, all patients provided informed consent. Flow cytometry was performed as described previously,[4] cells are presented per 100 000 counts. Laboratory tests were performed around the time of EPC studies. History of diabetes, hypertension, and hyperlipidemia was defined as any previous diagnosis of these conditions in the patient's charts before or at the time of EPC studies. Major adverse cardiovascular event was defined as any stroke, myocardial infarction, heart failure hospitalization, coronary revascularization, and all-cause death. Statistical analyses were performed using JMP or R software in a blinded manner.

After excluding patients with inadequate flow cytometry analyses (n=21) and who had acute coronary syndrome on the date of EPC measurement (n=28), a total of 997 patients were included in the analyses. The baseline characteristics are summarized in Figure [A]. Median CD34+, CD34+KDR+ (kinase insert domain receptor), and CD34+KDR+OC+ levels were 1010 (610–1530), 70 (20–203), and 14 (1–52) cells per 100 000 counts, respectively. CD34+ cell levels were lower in those with hypertension (940 [540–1490] versus 1040 [670–1620]; P=0.022), hyperlipidemia (940 [550–1480] versus 1080 [680–1630]; P=0.001), diabetes (880 [530–1373] versus 1030 [630–1610]; P=0.016), and tended to be lower in smokers (865 [515–1370] versus 1010 [620–1580] P=0.056). CD34+KDR+ cells were lower in those with a history of hyperlipidemia (50 [10,140] versus 80 [30–220]; P<0.001) and tended to be lower in those with hypertension (50 [10,160] versus 70 [20–170]; P=0.07). The fraction of OC-EPC was significantly higher in patients with cardiovascular risk factors (Figure [A] and [B]). These data suggested a potential procalcific shift in EPCs with cardiovascular risk factors. Considering kidney function as a risk factor for cardiovascular disease as well as calcification, the fraction of OC-EPCs showed an incremental increase with glomerular filtration rate fall (Figure [C]). Interestingly, we did not see any significant correlations between calcium or phosphorus levels and %OC-EPCs (R=0.04, P=0.47 for Ca, R=–0.12, P=0.06 for P), suggesting the association with renal function and the potential shift in EPC phenotypes could be due to other metabolites associated with decreased kidney function rather than mineral metabolism.

To assess the association between EPCs and future major adverse cardiovascular event risk, we investigated 903 patients with at least 6 months of follow-up. The median follow-up duration was 6.0 (3.2–9.0) years. In total, 309 patients had major adverse cardiovascular event, with 78 revascularizations, 53 myocardial infarctions, 108 heart failure hospitalizations, 56 cerebrovascular events, and 170 deceased. Higher than median CD34+ and CD34+KDR+ cell counts were associated with a lower risk of major adverse cardiovascular event during follow-up, although lost significance after

Key Words: coronary artery disease ■ endothelial progenitor cells ■ heart failure ■ hyperlipidemias ■ osteocalcin

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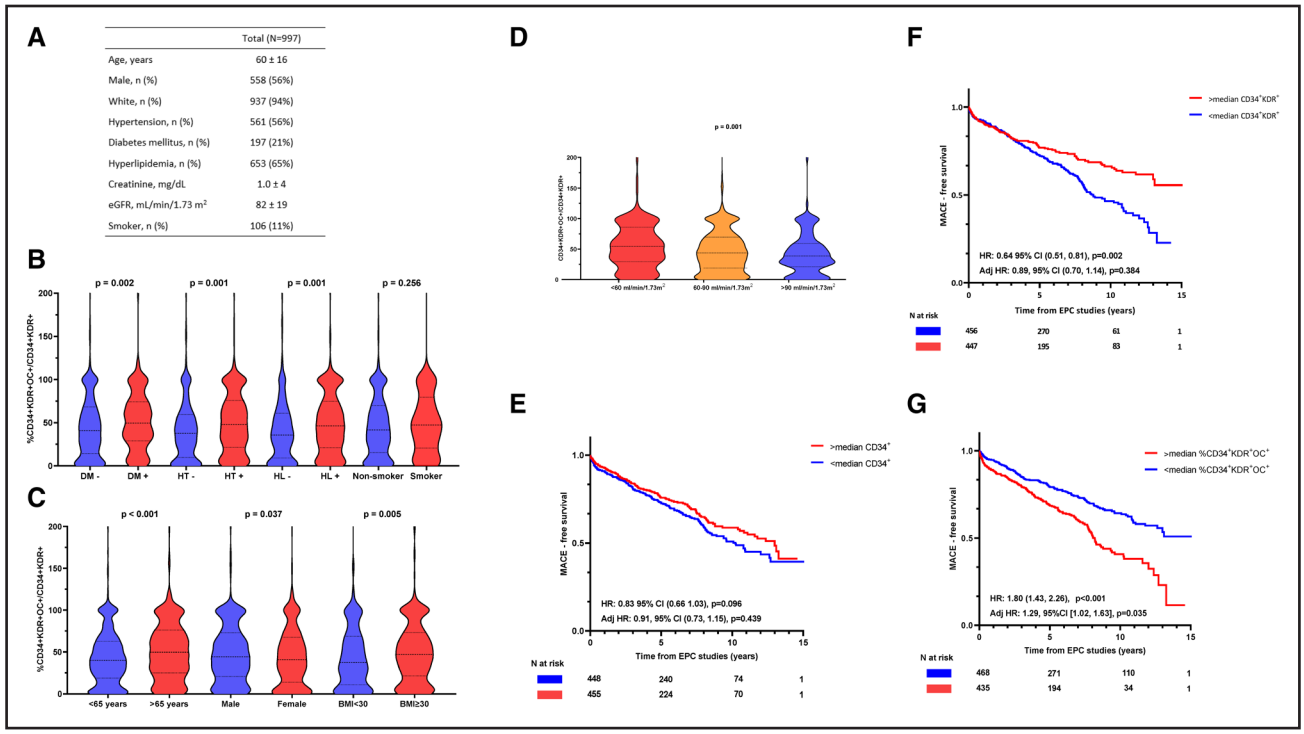

Figure. Association between endothelial progenitor cells (EPCs), cardiovascular risk factors and long-term prognosis. **A**, Baseline characteristics. **B**, Fraction of osteocalcin-expressing EPCs (OC-EPCs) was higher in patients with diabetes (DM; 51 [28–76] vs 42 [15–69]; *P*=0.002), hypertension (HT; 47 [22–76] vs 39 [12–62]; *P*=0.001), hyperlipidemia (HL; 45 [21–76] vs 38 [9–63]; *P*=0.001) whereas did not differ between smokers and nonsmokers (47 [21–81] vs 42 [18–71]; *P*=0.256). (Mann-Whitney *U* test). **C**, Fraction of OC-EPCs were higher in patients over 65 (48 [25–79] vs 39 [10–64]; *P*<0.001), males (44 [21–73] vs 41 [11–69]; *P*=0.037), and those with body mass index (BMI)>30 (47 [22–73] vs 38 [42–69]; *P*=0.005; Mann-Whitney U test). **D**, Four hundred seventy-eight (51%) had an estimated glomerular filtration rate (eGFR) between 60 and 90 mL/min, whereas 125 (13%) and 337 (36%) patients had GFR below 60 mL/min and above 90 mL/min, respectively. Fraction of OC-EPCs showed an incremental increase with GFR fall (56 [28–91] vs 46 [20–72] vs 38 [20–63], %; *P*=0.001). (Mann-Whitney *U* test) GFR was calculated using the CKD-EPI equation. **E**–**G**, Patients with greater than median levels of CD34+ and CD34+KDR+ (kinase insert domain receptor) cells have better long-term prognosis; however, this was not significant after adjusted for confounders (Adj) such as age, sex, HT, DM, HL, BMI, eGFR. In contrast, patients with higher fraction of OC-EPCs had worse prognosis even after adjustment for these mentioned confounders. Cox proportional hazard models and Kaplan-Meier curves were used for statistical analyses. HR indicates hazard ratio.

Nonstandard Abbreviations and Acronyms

EPC	endothelial progenitor cells
OC-EPC	osteocalcin-expressing EPCs

adjusted for risk factors of age, sex, hypertension, diabetes, hyperlipidemia, body mass index, and glomerular filtration rate. In contrast, higher OC-EPC levels, as well as a greater fraction of OC-EPCs, were associated with worse prognosis (Figure [D] through [F])

The current study provides significant preliminary findings regarding the role of OC-EPCs in cardiovascular health. EPC population is diverse, and our analysis suggests that one population that could have detrimental effects on cardiovascular health could be those that express osteocalcin. We suggest adding this marker for assessing EPCs might be useful in risk prediction. Furthermore, considering the recent therapeutic advances using progenitor cells in cardiovascular diseases,[5] preinfusion purification of a specific EPC population without osteocalcin expression might improve treatment outcomes. Our study is limited due to its retrospective design and should be considered hypothesis-generating; future studies are required to see whether they translate into outcomes.

ARTICLE INFORMATION

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Disclosures

None.

Data Availability

Data is available on request from the corresponding author.

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