# Circulating immature osteoprogenitor cells and arterial stiffening in postmenopausal osteoporosis

M. Pirro*, G. Schillaci, M.R. Mannarino, A.M. Scarponi, M.R. Manfredelli, L. Callarelli, C. Leli, G. Fabbriciani, R.S. Helou, F. Bagaglia, E. Mannarino

*Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Clinical and Experimental Medicine, University of Perugia, Hospital ''Santa Maria della Misericordia'', Piazzale Menghini, 1, 06129 Perugia, Italy*

**Abstract** *Background and aims:* An increased number of circulating osteoprogenitor cells (OPCs) expressing bone-related proteins and the stem cell marker CD34 have been identified in women with postmenopausal osteoporosis, who also have stiffer arteries than nonosteoporotic subjects. We investigated whether an increased number of circulating OPCs underlies the association of osteoporosis with arterial stiffness.

*Methods and results:* The number of circulating OPCs was quantified by FACS analysis in 120 postmenopausal women with or without osteoporosis. OPCs were defined as CD34+/alkaline phosphatase(AP)+ or CD34+/osteocalcin(OCN)+ cells. Participants underwent cardiovascular risk factor assessment, measurement of bone mineral density (BMD), and aortic pulse wave velocity (aPWV) as a measure of arterial stiffness.

Osteoporotic women had higher aPWV ($9.8 \pm 2.8$ vs $8.5 \pm 1.9$ m/s, $p = 0.005$) and levels of CD34+/AP+ and CD34+/OCN+ cells than nonosteoporotic controls [1045 $n$/mL (487—2300) vs 510 $n$/mL (202—940), $p < 0.001$; 2415 $n$/mL (1225—8090) vs 1395 $n$/mL (207—2220), $p < 0.001$]. aPWV was associated with log-CD34+/AP+ ($r = 0.27$, $p = 0.003$), log-CD34+/OCN+ cells ($r = 0.38$, $p < 0.001$). In stepwise regression analysis CD34+/OCN+ cells, age, systolic blood pressure and heart rate were significant predictors of aPWV (Model $R = 0.62$, $p < 0.001$), independent of cardiovascular risk factors, parathyroid hormone levels and osteoporotic status.

*Conclusion:* In women with postmenopausal osteoporosis an increased availability of circulating osteoprogenitor cells has a detrimental influence on arterial compliance, which may in part explain the association between osteoporosis and arterial stiffening.
© 2010 Elsevier B.V. All rights reserved.

* Corresponding author. Tel.: +39 075 5784024; fax: +39 075 5784022.
  *E-mail address:* mpirro@unipg.it (M. Pirro).

## Introduction

A few studies in humans reported the possible contribution of circulating osteoprogenitor cells (OPCs) to bone formation [1,2]. Accordingly, OPCs expressing bone-related proteins like alkaline phosphatase (AP) and osteocalcin (OCN) participate to osteogenesis in the early stage of fracture healing [3] and form mineralized bone in vitro and in vivo [1]. Other than bone-related proteins, circulating OPCs may also express CD34 [4,5], a cell-surface protein that has been widely used as a stem cell marker [6] for the hematopoietic and endothelial lineage. CD34+ cells may also differentiate into osteoblasts [5,7]; in addition, circulating CD34+ cells enhance vasculogenesis and osteogenesis and lead to functional recovery from bone fractures [8,9]. Hence, coexpression of CD34 and bone-related antigens allows to identify a population of cells with stem properties which may differentiate into endothelial and osteogenic cells. Since CD34+ progenitor cells home to vascular wall after endothelial injury [10] and CD34 confers a degree of plasticity to cells expressing this antigen [6], it has been hypothesized that CD34+ cells expressing also bone-related proteins may contribute to arterial calcification and possibly to atherosclerosis [4,5].

Arterial stiffness is a widely accepted early marker of arterial calcification [11,12], systemic atherosclerosis and cardiovascular risk [13,14]. Interestingly, both arterial calcifications and arterial stiffening have been observed in women with postmenopausal osteoporosis [15,16]. Although conventional wisdom holds that large artery stiffness mainly arises from the detrimental influence of major cardiovascular risk factors on endothelium integrity and function [17−20], other emerging mechanisms involving circulating OPCs may be supposed in osteoporotic women. The latter hypothesis is only partially supported by evidence showing an increased number of circulating cells coexpressing CD34 and bone-related proteins both in osteoporotic women [21] and in patients with coronary atherosclerosis [5]. Thus, whether increased number of circulating OPCs expressing both CD34 and bone-related proteins may underlie the association of postmenopausal osteoporosis with arterial stiffness remains to be elucidated.

The purpose of the present study was to investigate whether the number of circulating OPCs expressing the bone-related proteins alkaline phosphatase and osteocalcin and the hematopoietic-endothelial surface antigen CD34 is related to the presence of arterial stiffness in postmenopausal osteoporosis independently of traditional cardiovascular risk factors.

## Methods

### Study subjects

The study population consisted of 75 consecutive female outpatients with newly diagnosed, never-treated postmenopausal osteoporosis, and 45 age- and sex-matched nonosteoporotic (BMD T-score $>$ −1) postmenopausal controls selected among women independent in daily living activities attending for screening for postmenopausal osteoporosis our Unit of Bone and Mineral Metabolism. These groups of patients extended the population that we have recently described [21]. Women were considered postmenopausal if they had not been menstruating for at least 1 year. Diagnosis of postmenopausal osteoporosis was based on the presence of a T-score $\leq$ −2.5 SD at either the lumbar spine, femoral neck or proximal femur in either the absence or presence of $\geq$1 self-reported or radiology-documented fragility fracture occurred at least six months before the study recruitment. Fragility fractures were defined when occurred without trauma or falling from a standing height or less. Exclusion criteria included history of chronic diseases, such as renal, hepatic, cardiac, and rheumatic diseases, current or prior use of drugs that could interfere with bone mass (i.e. glucocorticoids, anti-resorpptive drugs and hormonal replacement therapy), and history of traumatic fractures. A trained interviewer applied to each participant a questionnaire regarding age, ages related to menstrual history (menopause and menarche), smoking habits, familial history of hip fractures, personal history of fragility fractures, daily calcium intake, alcohol consumption, pattern of habitual physical activity, medical history, co-morbid diseases, and medication use. Information was also obtained by review of medical records and laboratory data. The study was approved by the local Ethics Committee and all participants gave their informed consent.

### Clinical evaluation and bone mineral density

All the determinations were made at the medical center at 8 am, with a room temperature between 21 and 23 °C, after a 13-h overnight fast. Height and weight were measured to the nearest 0.1 cm and 0.1 Kg respectively, subjects were wearing hospital gowns and had bare feet. Body mass index was calculated as weight in kilograms divided by height squared in meters. Brachial blood pressure was measured by a physician with a mercury sphygmomanometer after patients sat for 10 min or longer. The average of 3 measurements was considered for the analysis.

Areal BMD (g/cm$^2$; bone mineral content relative to projection area) was measured by DXA (Hologic Discovery W, Hologic Inc, Bedford, MA, USA) at the lumbar spine (L1−L4) and the proximal femur, with a coefficient of variation at our laboratory of 0.49 and 0.51%, respectively. Results for areal BMD were transformed to $T$-scores, calculated as the difference between the actual measurement and the mean value of healthy gender-matched adult controls divided by their standard deviation.

### Measurement of arterial stiffness

Aortic pulse wave velocity (aPWV) was determined using an automatic device, the SphygmoCor Vx system (AtCor, Sydney, Australia), as previously described [18−20]. It uses a single-lead ECG and a high-fidelity applanation tonometer to measure the pressure pulse waveform sequentially in 2 peripheral artery sites, one at the base of the neck for the common carotid artery and the other over the femoral artery. aPWV is calculated from measurements of pulse transit time and the distance between the 2 sites,

according to the following formula: PWV (m/s) = distance (m)/transit time (s). The numerator is the distance between the suprasternal notch and the femoral artery minus the distance between the carotid sampling site and the suprasternal notch; the denominator is the time interval between the systolic $R$ wave and the femoral systolic up-stroke minus the time interval between the systolic $R$ wave and the carotid systolic up-stroke. The distance between the 2 sites is measured using a standard compass system, thus avoiding having the measurement influenced by thoracic and abdominal profiles. An average of 10 different cardiac cycles on each site was used for the analysis. All measurements were performed by the same observer, who was blinded to whether participants were osteoporotic or not. The intra-observer coefficient of variation of aortic PWV measured in 50 healthy young volunteers was 5.1%.

## Biochemical assays

Total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol and glucose were determined by enzymatic-colorimetric method (Dimension Autoanalyzer; DADE Inc. Newark, NJ); LDL cholesterol was calculated by the Friedewald equation in all participants. Serum intact parathyroid hormone (PTH) levels were measured by an immunoenzymatic method (Access, Beckman Coulter Inc., CA, USA). RIA assay was used to measure serum 25-OH-vitamin D (DiaSorin Inc., MN, USA).

## Assay of circulating osteoprogenitor cells

Measurement of circulating osteoprogenitor cells was performed as previously described [21]. Briefly, mononuclear cells were isolated from platelet depleted peripheral venous blood by density centrifugation (Lymphoprep, Axis-Shield PoC AS, Oslo, Norway). Exclusion of non viable mononuclear cells was performed by staining with 7-ami-noactinomycin D (Beckman Coulter Inc., Fullerton, CA, USA). Freshly isolated mononuclear cells were incubated for 30 min at 4 °C in the dark with biotinylated antibody against human AP (R&D Systems, Minneapolis, USA), PC5-conjugated streptavidin (Beckman Coulter Inc., Fullerton, CA, USA), FITC-conjugated antibody against human CD34 (Beckman Coulter Inc., Fullerton, CA, USA), ECD-coniugated antibody against CD15 (Beckman Coulter Inc., Fullerton, CA, USA) and PE-coniugated antibody against intracellular OCN (R&D Systems, Minneapolis, USA), according to manufacturer's instructions. Anti-CD15 antibody was used to exclude contamination of isolated mononuclear cells with granulocytes. Isotype-identical antibodies at a concentration matched with specific antibodies served as controls (Beckman Coulter Inc., Fullerton, CA, USA). All the antibodies were titrated to achieve working concentrations. After incubation, quantitative analysis was performed on a Coulter Epics XL measuring 100,000 cells per sample. OPCs were defined by negative staining for CD15 and positive staining for anti-CD34 and anti-AP or double positive staining for anti-CD34 and anti-OCN. The number of circulating OPCs was calculated by multiplying the frequency of fluorescent-positive events in the gate of lympho-monocytes by the total lympho-monocyte count. OPC count in two separate blood samples for each participant (sub-sample of 30 subjects) was highly reproducible ($r = 0.93$; $p < 0.001$).

## Statistical analysis

SPSS statistical package, release 10.0 (SPSS Inc., Chicago, Ill) was used for all statistical analyses. Values are expressed as the mean $\pm$ SD or SEM. Independent-sample $t$ test and Wilcoxon rank-sum test were used to compare the study variables between osteoporotic patients and control subjects. Correlation analyses were performed using the Pearson's and Spearman's coefficients of correlations. Logarithmic transformation was used for nonparametric variables. Stepwise regression analysis was used to estimate prediction of aPWV by including the following independent variables in the model: age, smoking status, waist circumference, systolic blood pressure (or alternatively mean blood pressure), heart rate, glucose, cholesterol, triglycerides, PTH, osteoporotic status and alternatively the log-transformed count of CD34+/AP+ or CD34+/OCN+ cells. Standardized coefficients were calculated as a measure for the relative predictive value. Statistical significance was assumed if a null hypotesis could be rejected at $p = 0.05$.

## Results

The characteristics of 120 postmenopausal women, 75 with newly diagnosed never-treated osteoporosis and 45 age-matched nonosteoporotic controls, are summarized in Table 1. Subjects with osteoporosis had lower BMD, body mass index, waist circumference and 25-OH-vitamin D levels, and higher total and LDL cholesterol levels and aPWV than controls. Fig. 1 illustrates the number of circulating CD34+/AP+, CD34+/OCN+ (Panel A) and total CD34 cell count (Panel B); the number of CD34+/AP+, CD34+/OCN+ and total CD34+ cells was significantly higher in osteoporotic than nonosteoporotic women, with significant 51%, 42% and 27% differences, respectively (Fig. 1, Panels A and B). Fig. 2 shows the direct correlation of CD34+/AP+ and CD34+/OCN+ cells with aPWV (Panels A and B, respectively). Neither brachial PWV nor augmentation index was significantly related to the number of circulating OPCs. Total CD34+ cell count was not significantly associated with aPWV. Other correlates of aPWV included age ($r = 0.31$, $p < 0.001$), waist circumference ($r = 0.32$, $p = 0.001$), systolic blood pressure ($r = 0.37$, $p < 0.001$), diastolic blood pressure ($r = 0.35$, $p < 0.001$), heart rate ($r = 0.18$, $p = 0.05$), creatinine levels ($r = 0.19$, $p = 0.03$), PTH levels ($r = 0.33$, $p < 0.001$) (Fig. 3, panel A) and 25-OH-vitamin D ($r = -0.26$, $p = 0.005$) (Fig. 3, panel B).

In order to identify independent predictors for aPWV, we performed a stepwise regression analysis including the following independent variables in the model: age, smoking status, waist circumference, systolic blood pressure (or alternatively mean arterial pressure), heart rate, glucose, cholesterol, triglycerides, PTH, osteoporotic status and alternatively the log-transformed count of CD34+/AP+ (Model 1) or CD34+/OCN+ cells (Model 2). In Model 1, age,

**Table 1** Clinical and biological characteristics of 120 study participants.

| | Osteoporotic ($N = 75$) | Nonosteoporotic ($N = 45$) |
|---|---|---|
| Age, years | 65 ± 13 | 64 ± 7 |
| Body mass index, kg/m$^2$ | 24.9 ± 5.8 | 27.9 ± 6.6* |
| Waist circumference, cm | 87 ± 10 | 94 ± 12* |
| Smokers, % | 21 | 22 |
| Systolic BP, mmHg | 136 ± 21 | 142 ± 18 |
| Diastolic BP, mmHg | 78 ± 9 | 81 ± 11 |
| Heart rate, bpm | 68 ± 8 | 71 ± 10 |
| Total cholesterol, mg/dL | 229 ± 38 | 206 ± 29* |
| LDL cholesterol, mg/dL | 137 ± 34 | 114 ± 30* |
| HDL cholesterol, mg/dL | 71 ± 14 | 68 ± 18 |
| Triglycerides, mg/dL | 106 ± 40 | 123 ± 63 |
| Glucose, mg/dL | 89 ± 19 | 90 ± 13 |
| PTH, pg/mL | 64.9 ± 34.5 | 57.2 ± 24.6 |
| 25-OH-vitamin D, ng/mL | 14.8 ± 10.0 | 19.5 ± 9.4* |
| Proximal femur BMD, g/cm$^2$ | 0.70 ± 0.11 | 0.94 ± 0.10* |
| Proximal femur $T$-score, SD | −2.0 ± 0.9 | 0.06 ± 0.8* |
| Lumbar spine BMD, g/cm$^2$ | 0.70 ± 0.11 | 0.94 ± 0.11* |
| Lumbar spine $T$-score, SD | −3.2 ± 0.9 | −0.9 ± 1.0* |
| aPWV, m/s | 9.8 ± 2.8 | 8.5 ± 1.9* |

Values are mean ± SD. *$p < 0.05$ for comparison between osteoporotic and nonosteoporotic postmenopausal women. BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoproteins; PTH, parathyroid hormone; BMD, bone mineral density; aPWV, aortic pulse wave velocity.

systolic blood pressure and heart rate were the only independent predictors of aPWV (Model $R = 0.51$, $p < 0.001$); in Model 2, age ($\beta = 0.41$, $p < 0.001$), systolic blood pressure ($\beta = 0.25$, $p = 0.009$), heart rate ($\beta = 0.20$, $p = 0.02$), and log-transformed count of CD34+/OCN+ cells count ($\beta = 0.25$, $p = 0.008$) were all positively associated with aPWV (Model $R = 0.62$, $p < 0.001$). Forcing in Model 2 also CD34+/AP+, 25-OH-vitamin D, estradiol and creatinine levels did not influence the final result, being age, blood pressure and CD34+/OCN+ cells independently associated with aPWV. Same results where obtained when replacing systolic blood pressure with mean arterial pressure.

## Discussion

Increased arterial stiffness has been observed in osteoporotic postmenopausal women [16,22—24]. It is currently unsettled however whether reduced arterial distensibility in women with postmenopausal osteoporosis can be explained solely by traditional cardiovascular risk factors, such as aging, increased systolic blood pressure, and sedentary lifestyle [22—24].

The main findings of the present study are that postmenopausal osteoporotic women have both high aPWV and circulating immature OPCs than nonosteoporotic subjects, and also that the number of circulating OPCs is directly associated with aPWV. To our knowledge this is the first observation of an association between circulating OPCs and arterial stiffening in postmenopausal osteoporotic women. Hence, although circulating OPCs are believed to play a beneficial influence on bone by actively contributing to mineralization [1—4], it is also possible that circulating cells expressing both stem cell and osteogenic markers, like CD34, alkaline phosphatase and osteocalcin, may have

a role in the process of arterial stiffening. Some lines of evidence might support this hypothesis. First, CD34+ progenitor cells are able to home to vascular wall [10] and to differentiate into osteoblasts [5,7]. Second, circulating CD34+ cells enhance osteogenesis and are able to form mineralized nodules in vitro [5,8]. Finally, significant increases in the percentage of CD34+ cells costaining for osteocalcin were noted in patients with coronary atherosclerosis compared to control subjects [5]. Thus, it is possible to speculate that CD34+ cells, thanks to their plasticity [6] allowing them to contribute to both vasculogenesis and osteogenesis [8,9], might enter the artery wall and participate in the vascular calcification process, that in turn has been associated with both osteoporosis [24—26] and increased arterial stiffness [11,12]. This speculation needs however to be confirmed by direct demonstration of the ability of circulating immature OPCs to enter the arterial wall and contribute to vascular calcification. Although previous studies have confirmed the ability of either CD34+, AP+ or OCN+ cells to form mineralized nodules in vitro under osteoblastic differentiation conditions [1,5], this issue has not been tested in the present study and the possible effects on in vitro mineralization of co-culturing OPCs and endothelial cells as well. The latter issues, along with the study of any potential interaction between OPCs and endothelial cells in vitro, might possibly provide a mechanistic demonstration of the association between OPCs and arterial stiffness.

We found a significant association between arterial stiffness and OPCs expressing the CD34 surface antigen, but not with CD34-negative OPCs (results not shown). This finding might further support the notion that expression of CD34 among cells expressing either alkaline phosphatase or osteocalcin is crucial for the positive link between osteogenic cells and arterial stiffness. Another important issue is
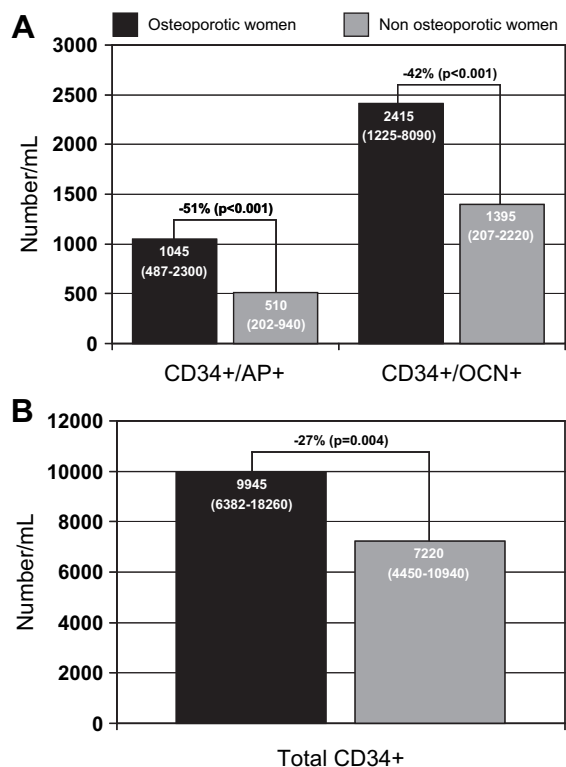
**Figure 1** Number per mL of circulating CD34+/AP+, CD34+/OCN+ (Panel A) and total CD34 cell count (Panel B) among 75 osteoporotic (black bars) and 45 nonosteoporotic postmenopausal women (grey bars). Data within parentheses indicate median and 25—75th percentile.

that the association between immature OPCs and aPWV was independent of the confounding effects of traditional covariates of arterial stiffness, like aging and increased systolic blood pressure, and also from other possible contributors to arterial stiffening in osteoporotic women, like high PTH levels and estrogen deficiency. Independence from these factors was tested in a multivartiate model, given the strong influence of traditional cardiovascular risk factors and PTH levels on arterial rigidity [17—20,27]. Since PTH may influence PWV, the presence of few osteoporotic women ($n = 17$) with PTH levels above the upper reference limit (88 pg/mL) secondary to hypovitaminosis D, might have contributed to increase PWV among the osteoporotic group compared to the control group, irrespective of OPC levels. However, it was not the case in the present study; indeed, association between OPCs and PWV remained highly significant even after correction for PTH levels; in addition, in a separate analysis in which women with PTH above 88 pg/mL were excluded, the association between OPCs and PWV was still significant.

In the present study, osteoporotic women had higher cholesterol levels than healthy controls which might have increased the degree of the association between OPCs and aPWV. However, osteoporotic women also had lower waist circumference and blood pressure levels, which might have lessened to a higher exent the degree of the association between OPC count and aPWV, being both waist circumference and systolic blood pressure positively associated with aPWV; in addition, neither in univariate and nor in

multivariate analyses, plasma cholesterol was significantly associated with aPWV.

Although simply quantifying peripheral OPCs does not explain the reason and the mechanism of their association with aPWV in postmenopausal osteoporosis, our findings might suggest the CD34+/OCN+ cell population as the cell lineage whose excess is closely related to bone mineral loss and arterial stiffening. The same cannot be claimed for CD34+/AP+ and total CD34 cell count. Hence, preferential expression of osteocalcin instead of alkaline phosphatase by circulating CD34 + OPCs might lead to a different interaction of these cells with the arterial wall. Moreover, total CD34+ cells were not significantly associated with aPWV, neither in bivariate correlations nor in multivariate regression analysis. This observation might simply reflect the great plasticity of peripheral CD34+ cells; accordingly, these cells are able to differentiate into cells influencing arterial compliance either positively, like endothelial progenitor cells [20], or negatively, like inflammatory cells [28] and osteoblast progenitors.

Limitations of our study need to be aknowledged. First, the observational design of the study does not allow us to reach conclusions on the mechanism underlying the association between OPCs and aPWV. In addition, the observational cross-sectional design itself, does not allow for the testing of hypotheses focused on the possible confounding
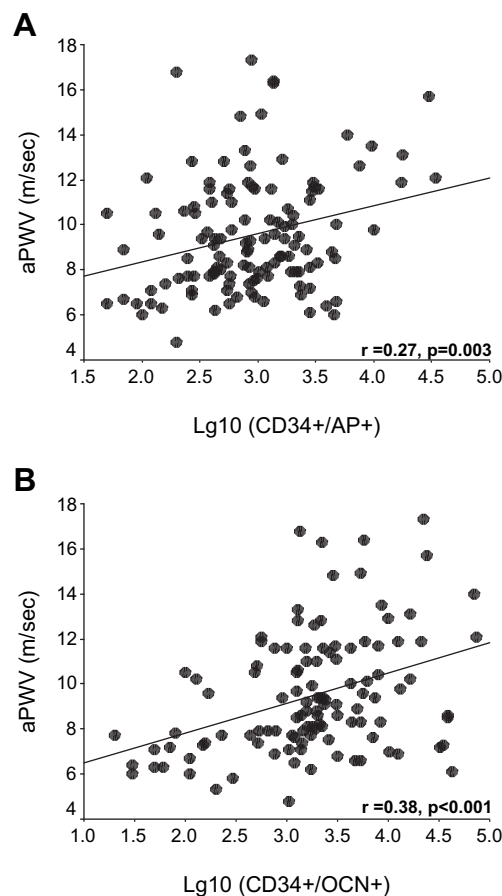


**Figure 2** Correlations of aortic pulse wave velocity (aPWV) with log-transformed CD34+/AP+ (Panel A) and CD34+/OCN+ osteoprogenitor cells (Panel B).

**A**



**B**
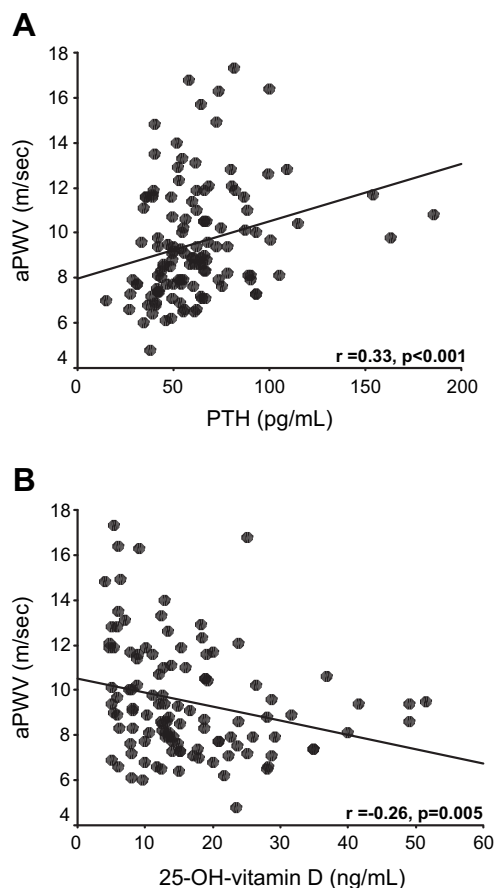


**Figure 3** Correlations of aortic pulse wave velocity (aPWV) with PTH (Panel A) and 25-OH-vitamin D levels (Panel B).

effects of current treatments on the study variables. It should be noted however that this was not a goal of the present study and that subjects exclusion criteria included current or prior use of drugs that could interfere with bone mass, like glucocorticoids, antiresorbptive drugs and hormonal replacement therapy.

Also, because of the cross-sectional design, data collection was limited to a single time point. Therefore, changes over time of the number of OPCs and aPWV were not assessed. Thus, independent OPC prediction of aPWV need to be validated also in prospective and intervention studies.

Second, a mechanistic confirmation of the present results was not provided in our study, that is mandatory before definitive conclusions are expressed on this issue. Our study, reported data merely on quantitative cytofluorimetric assessment of OPCs, but not on culture characteristics of these cells. Issues on vitality, differentiation and adhesion properties of cultured OPCs were not dealt within the present study; thus, limitations linked to quantitative cyto-fluorimetric assessment of circulating OPCs that is not sup-ported by qualitative culture analysis should be aknowledged. Further, measurement of the number of circulating endo-thelial progenitor cells might have provided additional mechanistic information on their relationship with OPCs.

In conclusion, in women with postmenopausal osteopo-rosis an increased availability of circulating immature

osteoprogenitor cells may have a detrimental impact on arterial compliance, which may in part explain the associ-ation between osteoporosis and arterial stiffening. A further understanding of the behaviour of OPCs in patients at increased cardiovascular and osteoporotic risk might provide additional knowledge on the emerging link between osteoporosis and atherosclerosis.

## Conflict of interest

## Funding sources

## Acknowledgments

## References

[1] Eghbali-Fatourechi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL, Khosla S. Circulating osteoblast-lineage cells in humans. N Engl J Med 2005;352:1959–66.

[2] Khosla S, Eghbali-Fatourechi GZ. Circulating cells with osteo-genic potential. Ann N Y Acad Sci 2006;1068:489–97.

[3] Kumagai K, Vasanji A, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model. J Orthop Res 2008;26:165–75.

[4] Eghbali-Fatourechi GZ, Mödder UI, Charatcharoenwitthaya N, Sanyal A, Undale AH, Clowes JA, et al. Characterization of circulating osteoblast lineage cells in humans. Bone 2007;40: 1370–7.

[5] Gössl M, Mödder UI, Atkinson EJ, Lerman A, Khosla S. Osteo-calcin expression by circulating endothelial progenitor cells in patients with coronary atherosclerosis. J Am Coll Cardiol 2008;52:1314–25.

[6] Nielsen JS, McNagny KM. Novel functions of the CD34 family. J Cell Sci 2008;121:3683–92.

[7] Chen JL, Hunt P, McElvain M, Black T, Kaufman S, Choi ES. Osteoblast precursor cells are found in CD34+ cells from human bone marrow. Stem Cells 1997;15:368–77.

[8] Matsumoto T, Kawamoto A, Kuroda R, Ishikawa M, Mifune Y, Iwasaki H, et al. Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing. Am J Pathol 2006;169:1440–57.

[9] Matsumoto T, Kuroda R, Mifune Y, Kawamoto A, Shoji T, Miwa M, et al. Circulating endothelial/skeletal progenitor cells for bone regeneration and healing. Bone 2008;43:434–9.

[10] Zampetaki A, Kirton JP, Xu Q. Vascular repair by endothelial progenitor cells. Cardiovasc Res 2008;78:413–21.

[11] Adragão T, Pires A, Birne R, Curto JD, Lucas C, Gonçalves M, et al. A plain X-ray vascular calcification score is associated with arterial stiffness and mortality in dialysis patients. Nephrol Dial Transplant 2009;24:997–1002.

[12] McEniery CM, McDonnell BJ, So A, Aitken S, Bolton CE, Munnery M, et al. Aortic calcification is associated with aortic

stiffness and isolated systolic hypertension in healthy individuals. Hypertension 2009;53:524—31.

[13] Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39:10—5.

[14] McEniery CM, Cockcroft JR. Does arterial stiffness predict atherosclerotic coronary events? Adv Cardiol 2007;44:160—72.

[15] Frost ML, Grella R, Millasseau SC, Jiang BY, Hampson G, Fogelman I, et al. Relationship of calcification of atherosclerotic plaque and arterial stiffness to bone mineral density and osteoprotegerin in postmenopausal women referred for osteoporosis screening. Calcif Tissue Int 2008; 83:112—20.

[16] Hirose K, Tomiyama H, Okazaki R, Arai T, Koji Y, Zaydun G, et al. Increased pulse wave velocity associated with reduced calcaneal quantitative osteo-sono index: possible relationship between atherosclerosis and osteopenia. J Clin Endocrinol Metab 2003;88:2573—8.

[17] Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. Hypertension 2005;45:1050—5.

[18] Pirro M, Schillaci G, Savarese G, Gemelli F, Vaudo G, Siepi D, et al. Low-grade systemic inflammation impairs arterial stiffness in newly diagnosed hypercholesterolaemia. Eur J Clin Invest 2004;34:335—41.

[19] Schillaci G, Pirro M, Vaudo G, Mannarino MR, Savarese G, Pucci G, et al. Metabolic syndrome is associated with aortic stiffness in untreated essential hypertension. Hypertension 2005;45:1078—82.

[20] Pirro M, Schillaci G, Paltriccia R, Bagaglia F, Menecali C, Mannarino MR, et al. Increased ratio of CD31+/CD42—

microparticles to endothelial progenitors as a novel marker of atherosclerosis in hypercholesterolemia. Arterioscler Thromb Vasc Biol 2006;26:2530—5.

[21] Pirro M, Leli C, Fabbriciani GL, Manfredelli MR, Callarelli L, Bagaglia F, et al. Association between circulating osteoprogenitor cell numbers and bone mineral density in postmenopausal osteoporosis. Osteoporos Int 2010;19:297—306.

[22] Sumino H, Ichikawa S, Kasama S, Takahashi T, Kumakura H, Takayama Y, et al. Elevated arterial stiffness in postmenopausal women with osteoporosis. Maturitas 2006;55: 212—8.

[23] Mangiafico RA, Alagona C, Pennisi P, Parisi N, Mangiafico M, Purrello F, et al. Increased augmentation index and central aortic blood pressure in osteoporotic postmenopausal women. Osteoporos Int 2008;19:49—56.

[24] Mikumo M, Okano H, Yoshikata R, Ishitani K, Ohta H. Association between lumbar bone mineral density and vascular stiffness as assessed by pulse wave velocity in postmenopausal women. J Bone Miner Metab 2009;27:89—94.

[25] Rajzbaum G, Bézie Y. Postmenopausal osteoporosis and atheroma. Jt Bone Spine 2006;73:661—6.

[26] Naves M, Rodríguez-García M, Díaz-López JB, Gómez-Alonso C, Cannata-Andía JB. Progression of vascular calcifications is associated with greater bone loss and increased bone fractures. Osteoporos Int 2008;19:1161—6.

[27] Rubin MR, Maurer MS, McMahon DJ, Bilezikian JP, Silverberg SJ. Arterial stiffness in mild primary hyperparathyroidism. J Clin Endocrinol Metab 2005;90:3326—30.

[28] Wakabayashi I, Masuda H. Association of acute-phase reactants with arterial stiffness in patients with type 2 diabetes mellitus. Clin Chim Acta 2006;365:230—5.