

CIRCULATING BONE MARROW DERIVED OSTEO- PROGENITORS IN VASCULATURE CONTRIBUTES TOWARDS VASCULAR CALCIFICATION

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Vascular calcification is an important determinant of cardiovascular mortality. The mechanisms responsible for vascular calcification are controversial. It has been suggested that the bone marrow derived osteo-progenitor population may contribute to the development of arterial mineralization. Osteocalcin expressing mononuclear cells (OCN+ MNCs) have recently been demonstrated within the circulation of adults and shown to have ability to carry out mineralization both in vitro and within mice. The aim of this study was to assess the association of vascular calcification with circulating bone marrow derived osteo-progenitors and stem cell mobilizing cytokines in two mice models and a human patient cohort.

We investigated a number of mice models including one dependent on calcitriol injection in osteoprotegerin deficient (OPG^{-/-}) mice (n=12). The severity of aortic calcification in the aortas of 12 month old male (n=10) and female mice (n=10) was evaluated using alizarin red staining and a bioassay. Tail bleeds (n=160) were analyzed for OCN+ MNCs using flow cytometry. We also estimated the percentage of circulating OCN+ MNCs using flow cytometry in patients (n=23) suffering from peripheral artery diseases. Infra renal aortic calcification volume was measured in patients by computed tomography. Stem cell mobilizing cytokines such as stromal cell derived factor (SDF-1 α), granulocyte colony stimulating factor (G-CSF) and stem cell factor (SCF) were investigated in patient plasma samples. A three way correlation was assessed for infra renal calcification volumes, circulating OCN + MNCs and these cytokines.

Comparative studies showed that the percentage of circulating OCN+ MNCs was significantly more in mice with aortic calcification than control animals. The severity of aortic calcification was correlated to the number of circulating OCN+ MNCs (r=0.525 for male and 0.563 for female mice). When aortic calcification was accelerated using calcitriol similar associations were demonstrated in younger mice (r= 0.641 in OPG^{-/-} with calcitriol vs. r=0.525 in older OPG^{-/-} without calcitriol) In human study, Patients with more severe aortic calcification (calcification volume \geq median) had a greater percentage of circulating OCN+ MNCs (median 4.07 %, IQR 3.76-4.39, n=12) than those with less severe aortic calcification (median 3.10 %, IQR 2.32-3.60, n=11, p=0.05). The OCN+ MNC percentage was moderately correlated (r=0.475, p=0.22) with aortic calcification volumes. Similarly, plasma SDF-1 α , G-CSF and SCF concentrations were also observed to be significantly associated with the OCN+ MNC percentage (0.597, p=0.03; 0.654, p=0.001; 0.654, p=0.003 respectively) and calcification volumes (0.480, p=0.01; 0.522, p=0.001; 0.480, p=0.01 respectively)

This study demonstrates, for the first time, that circulating bone marrow derived osteo-progenitors can be robustly quantified in murine as well as patient cohort with varied degree of aortic calcification. OCN+ MNC were associated with the severity of aortic calcification. Overall our findings have implications for bone marrow based cellular therapies being developed.