

INVESTIGATIVE REPORT

Decreased Number of Circulating Endothelial Progenitor Cells (CD133⁺/KDR⁺) in Patients with Psoriatic Arthritis

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Cardiovascular diseases are a major cause of mortality in patients with psoriatic arthritis (PsA), but the precise mechanism of increased cardiovascular risk is unknown. Endothelial dysfunction plays a crucial role in the development of atherosclerosis. Circulating endothelial progenitor cells (CEPCs) contribute to endothelial regeneration and their level may be affected by chronic inflammation. The aim of this study was to evaluate the number of CEPCs in patients with PsA ($n=24$) compared with controls ($n=26$). CEPCs were identified as CD133⁺/KDR⁺ cells in peripheral blood, using flow cytometry. A significantly decreased number of CEPCs was observed in patients with PsA ($p<0.0001$). The number of these cells was significantly, inversely correlated with the severity of skin and joint involvement (Psoriasis Area and Severity Index (PASI), DAS28) and the level of C-reactive protein. We hypothesize that the reduced number of CEPCs may indicate and contribute to the increased cardiovascular risk in patients with PsA. Key words: psoriatic arthritis; circulating endothelial progenitor cells; cardiovascular risk; atherosclerosis; endothelial dysfunction; inflammation.

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Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory disease, classified as a seronegative inflammatory arthropathy and characterized by the association of arthritis and psoriasis. The estimated prevalence of PsA among patients with psoriasis ranges widely from 7.7% to 73%, with the majority of studies reporting the presence of PsA in approximately 10–30% of psoriatic individuals (1–3). Most patients with PsA present signs of skin or nail disease before experiencing symptoms of arthritis. Although PsA disease activity is commonly not mirrored by the skin disease, PsA is more prevalent in patients with more severe skin involvement (2, 3). PsA is a complex and heterogeneous disease that may have variable severity, clinical manifestation and course

with possible involvement of the peripheral and/or axial joints, periarticular structures, skin and nails. The most common manifestation of PsA is peripheral joint involvement (1–5).

In addition to involvement of skin or joints, both psoriasis and PsA are associated with numerous comorbidities. Cardiovascular diseases (CVD) and cardiovascular events (CVE), such as ischaemic heart disease, myocardial infarction, and stroke, represent a major cause of morbidity and mortality in patients with PsA (6–8). Furthermore, it has been shown that patients with PsA have higher cardiovascular risk and more severe subclinical atherosclerosis compared with patients with plaque psoriasis (6, 9–11). However, the pathomechanism underlying this observation is not completely understood.

Circulating endothelial progenitor cells (CEPCs) constitute a population of bone marrow-derived cells in peripheral blood that have the capacity to migrate, proliferate and differentiate into mature endothelial cells. CEPCs play a significant role in endothelial regeneration and maintenance of vascular homeostasis. Therefore, a reduced number of CEPCs has been shown to significantly contribute to the development of atherosclerosis and to constitute a biomarker for increased cardiovascular risk (12–19). Furthermore, CEPCs contribute to neovascularization, in a process termed postnatal vasculogenesis (17, 18). Impaired number and function of CEPCs have been found in patients with cardiovascular risk factors, CVD and some chronic inflammatory diseases (16, 18–22).

Our group has previously assessed the number of CEPCs in patients with plaque psoriasis (22), and found a significantly reduced number of CEPCs in these patients compared with controls. Moreover, we demonstrated a significant inverse correlation between the number of these cells and the severity of skin involvement, assessed with the Psoriasis Area and Severity Index (PASI). We hypothesized that the reduced number of CEPCs may result from a systemic inflammatory process and contribute to the increased cardiovascular risk in patients with plaque psoriasis (22). In the current study we attempted to evaluate the number of CEPCs in patients with PsA. We also evaluated the level of vascular endothelial growth factor (VEGF), a potent

proangiogenic factor, to investigate its potential association with the number of CEPCs.

MATERIALS AND METHODS (for complete details see Appendix S1¹)

The study was approved by the local Bioethical Committee according to the principles of the Declaration of Helsinki (No. 283/2008 and No. 672/2012). Twenty-four subjects with PsA and 26 controls with no history of psoriasis or any other skin disease, matched by age and sex, were recruited to the study. Demographic data as well as information regarding the presence of conventional cardiovascular risk factors (hypertension, diabetes, obesity, smoking habit) and medication use were documented. The diagnosis of PsA was confirmed by the Classification Criteria for Psoriatic Arthritis (CASPAR) (23). The baseline evaluation included disease activity score (DAS) 28 and peripheral joint assessment (68 joints for tenderness and 66 joints for swelling). All patients had peripheral type of PsA; either asymmetrical oligoarthritis or symmetrical polyarthritis, according to the Moll & Wright classification (24). The severity of skin involvement was assessed with the PASI. The number of CEPCs was assessed with flow cytometry using a FACS-Calibur cytometer (Becton Dickinson, New Jersey, USA). The CEPCs were determined as CD133⁺, VEGFR-2/KDR⁺ cells. Fluorescence-activated cell sorting (FACS) analysis was performed using CellQuest software (Becton Dickinson). VEGF was measured by enzyme-linked immunoassay (ELISA) using a commercially available kit (Quantikine Human VEGF Immunoassay, R&D Systems, Minneapolis, MN, USA, cat. DVE00), according to the manufacturer's instructions. Statistical analysis was performed using the software Statistica version 9.0 (Windows XP) and GraphPad Prism version 5.0.

RESULTS

The number of CEPCs was significantly decreased in patients with PsA compared with controls (295.9 ± 228 cells/ml and 687.7 ± 361 cells/ml, respectively; $p < 0.0001$) (Fig. 1). There were no significant correlations between the number of CEPCs and demographic or clinical factors, such as age, sex, or duration of psoriasis. The number of CEPCs was significantly correlated with the severity of joint involvement assessed

with DAS28 ($p = 0.0028$, $R = -0.67$) (Fig. 2). Furthermore, there were significant correlations between the number of CEPCs and the severity of skin involvement, assessed with PASI ($p = 0.035$, $R = -0.51$) and the number of CEPCs and the level of C-reactive protein (CRP) ($p = 0.009$, $R = -0.60$) (Fig. 2). Compared with previously published data concerning patients with plaque psoriasis (22), the number of CEPCs was significantly lower in individuals with PsA ($p = 0.042$) (Fig. 1). There were no statistically significant differences regarding age, sex or incidence of assessed cardiovascular risk factors, such as obesity, hypertension, or smoking habit, between patients with plaque psoriasis and those with PsA (Table S1¹). The level of VEGF was significantly elevated in psoriatic patients compared with controls (188.6 ± 127.6 pg/ml and 94.45 ± 47.77 pg/ml, respectively; $p = 0.044$). No significant correlation was found between the level of VEGF and the number of CEPCs.

DISCUSSION

The current study found a significantly decreased number of CEPCs (CD133⁺/KDR⁺) in patients with PsA compared with controls. Furthermore, the number of CEPCs in patients with PsA was inversely correlated with measures of disease activity, such as severity of joints and skin involvement (DAS28, PASI) as well as with the systemic inflammatory marker CRP. Numerous studies have demonstrated the increased cardiovascular risk in patients with psoriasis. Higher prevalence of cardiovascular risk factors, such as hypertension, obesity or metabolic syndrome, has been found in patients with psoriasis. However, the increased cardiovascular risk was also demonstrated in individuals with psoriasis without any conventional cardiovascular risk factors (7, 8, 25–27). There is growing evidence that chronic inflammation contributes to the development of endothelial dysfunction and atherosclerosis in psoriasis. However, a pathomechanistic link has not been found (12, 28, 29). Atherosclerosis is a chronic inflammatory disease of the vessel wall with endothelial dysfunction playing an important role (12, 30). CEPCs play a significant role in the protection and regeneration of endothelium. A reduced level of these cells has been suggested to constitute an independent cardiovascular risk factor and biomarker for endothelial dysfunction and increased cardiovascular risk (14–18). Various inflammatory mediators, such as CRP, as well as traditional cardiovascular risk factors, such as smoking habit, obesity, and hypertension, seem to affect the number of CEPCs (17–19, 31). Previously, our group observed a reduced number of CEPCs (CD133⁺/KDR⁺) in patients with plaque psoriasis compared with controls, matched by age, sex and major cardiovascular risk factors. The number of these cells was inversely correlated with severity of

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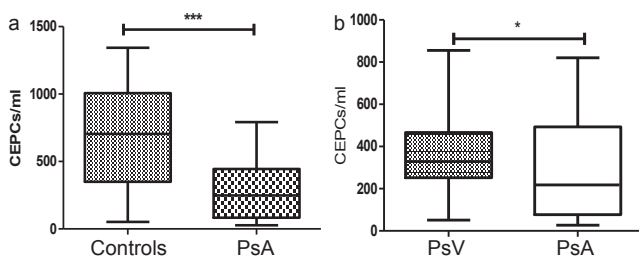


Fig. 1. (a) Decreased number of circulating endothelial progenitor cells (CEPCs) in patients with psoriatic arthritis (PsA) ($n = 24$) compared with controls ($n = 26$) ($p < 0.0001$). (b) Decreased number of CEPCs in patients with PsA ($n = 24$) compared with patients with plaque psoriasis (psoriasis vulgaris; PsV) ($n = 63$)^a ($p = 0.042$). ^aData published previously (22).

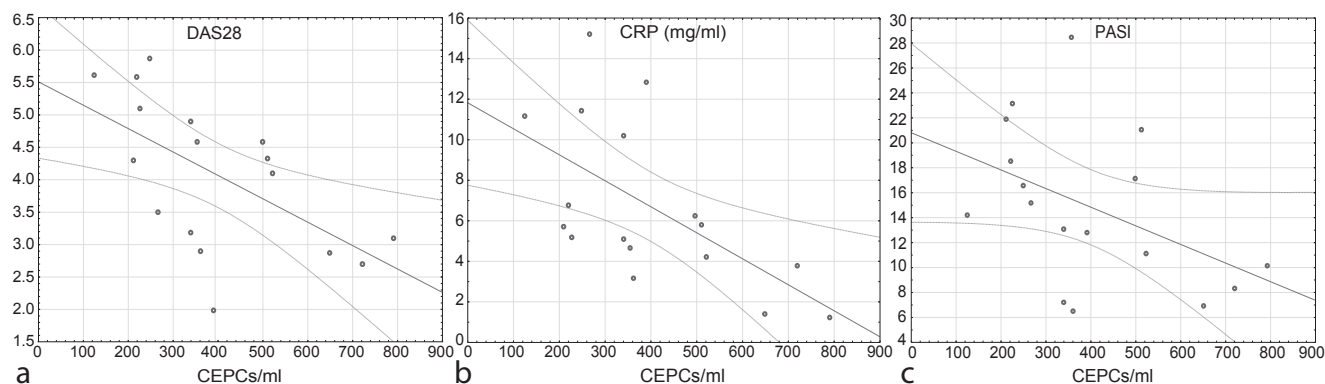


Fig. 2. Correlations between the number of circulating endothelial progenitor cells (CEPCs) and measures of disease activity in patients with psoriatic arthritis (PsA). (a) Disease activity score 28 (DAS28) ($p=0.0028$, $R=-0.67$). (b) C-reactive protein (CRP) ($p=0.009$, $R=-0.60$). (c) Psoriasis Area and Severity Index (PASI) ($p=0.035$, $R=-0.51$).

skin involvement (22). Compared with patients with plaque psoriasis, individuals with PsA have a higher cardiovascular risk (7–10). A higher prevalence of subclinical atherosclerosis, which was independent of traditional cardiovascular risk factors, was found in patients with PsA compared with those with plaque psoriasis (10). It is postulated that increases in inflammatory pathways in PsA may contribute to a higher cardiovascular risk compared with plaque psoriasis (10). However, the pathomechanism that links the inflammatory process and atherosclerosis in PsA is not completely understood. Using the same methodology as in a previous study (22), we found a significantly decreased number of CEPCs ($CD133^+/KDR^+$) in patients with PsA compared with controls. Furthermore, the number of CEPCs was lower in patients with PsA compared with patients with plaque psoriasis (data previously published, 22), matched by age, sex and major cardiovascular risk factor. Increased levels of numerous proinflammatory mediators have been found in the systemic circulation in patients with psoriasis. The intensity of the systemic inflammatory process in PsA is probably dependent on the severity of skin and joint involvement (32). The correlations between disease severity (DAS28, PASI, CRP) and the numbers of CEPCs, demonstrated in our study, may point to the negative impact of the inflammatory process on the number of CEPCs. On the other hand, the presence of a cardiovascular risk factor might also affect the number of these cells. The reduced number of CEPCs may also result from the contribution of these cells to the process of neoangiogenesis in psoriasis (20, 33).

To the best of our knowledge, there is only one publication concerning the number of CEPCs in patients with PsA. Ablin et al. (34) found no statistically significant difference in the number of CEPCs between patients with PsA ($n=22$) and controls ($n=16$). CEPCs were identified as $CD133^+/CD34^+$ and $CD34^+/KDR^+$ cells in the peripheral mononuclear cell fraction using flow cytometry (34). It should be noted that levels of $CD34^+/KDR^+$ cells were lower in patients with PsA compared

with controls, although not significantly. Contrary to Ablin et al. (34), we used another combination of surface markers that probably identifies a different subpopulation of CEPCs with potent vasoregenerative potential (15). Moreover, different characteristics of the study groups may influence the results. In the study of Ablin et al. (34), the frequency of a smoking habit in the control group was significantly higher than in the group of patients, which might have influenced the number of CEPCs. Moreover, a substantial number of patients with PsA ($n=12$) were also previously treated with tumour necrosis factor alpha (TNF- α) antagonists (34). A decreased number of CEPCs and a beneficial effect of TNF- α antagonists on CEPC count and vascular function in patients with plaque psoriasis have been reported recently (35, 36).

VEGF is a potent proangiogenic mediator that influences migration, homing and proliferation of CEPCs (14, 37). We found significantly increased serum levels of VEGF in patients with PsA compared with controls. This may point to the existence of a certain compensatory mechanism for the decreased number of CEPCs in PsA, although we did not detect any correlation between the level of this factor and the number of CEPCs.

There are some controversies about the precise phenotypic definition of CEPCs and thus their function and significance as a biomarker for cardiovascular disease. This hampers translation of basic research into clinical use (38, 39). Despite the considerable debate, CEPCs are often quantified with flow cytometry as double positive for progenitor markers ($CD133$, $CD34$) and the endothelial marker KDR ($VEGFR-2$). Different combinations of these markers seem to characterize subpopulations of CEPCs at different maturation levels and with different functions (15, 38, 39). Nevertheless, $CD133^+/KDR^+$ cells have been shown to possess substantial vasoregenerative potential (15, 40). It will be interesting to conduct future studies regarding the exact role of CEPCs ($CD133^+/KDR^+$) in the pathogenesis of psoriasis comorbidities and regulation of these cells, e.g. by various proinflammatory markers. The com-

parison of psoriatic patients with CVD and without any cardiovascular risk, in patients before and after treatment, would be an interesting approach for future research regarding this issue.

The decreased number of CEPCs in patients with PsA may both indicate and contribute to increased cardiovascular risk. It may be hypothesized that the reduced number of these cells may constitute the link between inflammatory process, presence of cardiovascular risk factors, endothelial dysfunction and increased cardiovascular risk in patients with PsA. However, future studies are necessary to investigate the precise role of these cells in the pathogenesis of psoriatic cardiovascular comorbidities, as well as to establish their potential use in clinical practice.

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