

# Circulating Endothelial Cells in Vasculitis and Transplantation

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## Key Words

Circulating endothelial cells · Vasculitis

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## Circulating Endothelial Cells in Vasculitis

Damage to microvascular endothelial cells is the hallmark of small-vessel vasculitis. Based on the histology, however, an intriguing question has long been unanswered: Given that cells undergo detachment from the basement, what happens to these cells and can we detect them in peripheral blood? Moreover, it seemed reasonable to assume that the number of circulating endothelial cells would reflect disease extent and activity. For this reason, we became interested in circulating endothelial cells as a possible marker of disease activity in ANCA-associated vasculitis. In our study [1], high numbers of cells (>100/ml) were detected in patients with active systemic vasculitis; cell numbers declined progressively during the course of successful immunosuppressive treatment. Moderately elevated numbers of circulating endothelial cells were detected in blood obtained from patients in remission. Finally, controls with infection and non-vasculitic renal disease did not have elevated cell numbers. Therefore, the positive predictive value of a cell count above 25 cells/ml was 100% while the nega-

tive predictive value was 97%. We concluded that circulating endothelial cells are new markers of ANCA-associated vasculitis, may be of vasculitis in general.

Interestingly, a necrotic phenotype as evidence of the severity of the inflammatory process and tissue-factor expression of the cells could be demonstrated. This finding supports the notion that tissue factor [2] may be an important link between inflammation and coagulation. Tissue-factor positive endothelial microparticles have also been described in sickle cell anemia [3] and in vitro [4]. Induction of tissue factor by ANCA and by proteases released during leukocyte activation in ANCA-associated vasculitis has been demonstrated in vitro [5,6]. Fibrin, a typical feature of vasculitis lesions, induces tissue factor in endothelial cells [7] while tissue factor, in turn, induces fibrin deposition [8].

One limitation of our study [1] was that only patients with ANCA-associated small-vessel vasculitis had been included. In contrast, data regarding other forms of vasculitis are sparse at present. Dang and co-workers demonstrated elevated numbers of circulating endothelial cells as well as enhanced levels of endothelin in large-vessel vasculitis [9] correlating with disease activity. Nakatani et al have reported similar findings in Kawasaki's disease [10]. In 20 patients they demonstrated elevated numbers of circulating endothelial cells. Patients with coronary artery lesions had significantly higher cell numbers than patients without suggesting an association with disease activity.

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Another limitation of our study [1] was the lack of patients with relapse. We have meanwhile gathered more experience with the use of this marker in this regard. In our experience, a rise of circulating endothelial cells is observed in patients who relapse although the elevation is rather moderate and protracted (unpublished data). Another application is the use of circulating endothelial cells as a screening test whenever other tests do not suffice. We use this marker as an adjunctive tool similar to ANCA measurements. It must be emphasized, however, that both augment rather than replace clinical assessment.

### Circulating Endothelial Cells in Transplantation

The transplant field offers another exciting opportunity to study vascular disease. It is well known that renal transplant recipients have a high incidence of vascular complications. We were able to demonstrate elevated numbers of circulating endothelial cells in renal transplant recipients [11]. Patients who receive cyclosporine A as part of their immunosuppressive regimen have higher cell numbers than renal transplant recipients who do not receive this drug [12]. These findings lend further support to the hypothesis that calcineurin inhibitors damage microvascular endothelial cells. It remains to be shown whether other transplant recipients and patients on tacrolimus, another calcineurin inhibitor, also have elevated cell numbers. Moreover, studies in patients who receive such drugs for indications other than transplantation as well as monitoring of cell numbers before and after stoppage of such drugs would be worthwhile.

We conducted another study in haematopoietic stem-cell transplantation (HSCT). We have, for the first time, demonstrated elevated numbers of circulating endothelial cells in allogeneic HSCT [13]. After conditioning, cell numbers were significantly elevated (median 44 cells/ml) compared to baseline (median 16 cells/ml) and controls (median 8 cells/ml). Patients with reduced intensity conditioning had significantly lower cell numbers (median 24 cells/ml) than those who received standard conditioning. Patients who received TBI tended to have a brisk rise in cell numbers while patients who received chemotherapy had more protracted elevation. These findings, we believe, are well in line with current concepts of radiation-induced endothelial damage [14]. Interestingly, cell numbers prior to conditioning were not normal but slightly elevated when compared to healthy controls. This finding led us to speculate as to which events prior to conditioning could be responsible. In this regard, modestly elevated numbers of circulating endothelial cells in patients with various tumours has recently been documented [15]. In this setting, they may reflect endothe-

lial apoptosis during tumour angiogenesis [14]. Alternatively, elevated cell numbers prior to conditioning could reflect endothelial damage accrued by previous radiation or chemotherapy.

Moreover, we were interested to note high variability of cell numbers. We assume that patients display different degrees of vulnerability for the effects of irradiation and chemotherapy. It is conceivable that pre-existing atherosclerosis and age play a role although we did not test this hypothesis so far. Moreover, endothelial apoptosis during irradiation depends on a variety of factors, such as the nutritional factors, hypoxia. Finally, a genetic background of vulnerability has been suggested [14,16].

In summary, our findings yield a novel marker to evaluate the extent of endothelial damage during various conditioning regimens.

### Pathophysiology of Circulating Endothelial Cells

One enigma about circulating endothelial cells is their way of detachment from the basement membrane. It is well documented that shear stress suppresses endothelial cell apoptosis by virtue of several pathways, such as up-regulation of nitric oxide synthase [17]. In addition, mechanisms of endothelial detachment in health and disease remain enigmatic. In inflammatory disorders, various factors such as direct neutrophil attack, cytokines and proteases may be at play. In contrast, interaction with neighbouring cells and anchorage to extracellular matrix, possibly mediated by vitronectin, fibronectin, cadherins, as well as integrins are crucial to endothelial cell survival. At this point, questions remain. Do these cells disintegrate into smaller particles in situ or after they have been detached from the basement membrane? One would also be curious as to the fate of circulating endothelial cells: Is there a clearance mechanism, for example in liver, spleen, or pulmonary capillaries? Sophisticated studies would be necessary to elucidate such mechanisms. One approach in vasculitis could be selective sampling of venous blood from affected tissues and comparison of cell numbers with mixed venous blood and blood from unaffected tissues.

Another more hypothetical question is whether circulating endothelial cells might be capable of causing an inflammatory response in their own right. Release of substances by circulating necrotic endothelial cells is one pathway by which these cells may exert gain further importance. High mobility group 1 (HMGB1) protein is a protein that is released from necrotic cells. After release, HMGB1 binds to RAGE (the receptor for advanced glycation end products) and acts as a potent mediator of inflammation [18]. Other

proteins, such as cytochromes, are also released although their significance remains unclear [19]. Plasma DNA released from necrotic endothelial cells [20] may also cause secondary phenomena. Finally, release of heat shock proteins from necrotic cells has been shown to deliver a maturation signal to dendritic cells [21]. All these proteins may be released from necrotic circulating endothelial cells with a whole array of pathophysiologic consequences. It must be emphasized, however, that such an assumption is entirely hypothetical at present.

There is another pathway by which necrotic circulating endothelial cells could gain pathophysiologic significance. It has been shown previously that cells, such as fibroblasts, are able to sense the presence of necrotic cellular debris in their vicinity [22]. This study demonstrated, for the first time, that necrotic but not apoptotic cells initiate a Toll-like-receptor-2/NF B-dependent reaction in monocytes and fibroblasts. Moreover, uptake of necrotic cellular material

activates macrophages [23]. It is conceivable, but entirely speculative, that healthy endothelial cells or circulating leukocytes react to the presence of necrotic endothelial debris in a similar manner. To address this issue, we are currently exposing human umbilical vein endothelial cells with necrotic endothelial cells. Preliminary data suggest that healthy HUVEC do indeed react to the presence of necrotic endothelial cells with a doubling in their interleukin-6 production (unpublished data). Internalization of necrotic material by macrophages is also well documented [24]. Recent evidence suggests that mannose-binding lectin [25] and surface phosphatidylserine [26] are involved in this process. Presumably, necrotic endothelial debris undergoes similar mechanisms, which may, in turn, induce other inflammatory signals.

In summary, pathophysiologic effects of circulating endothelial cells are a fascinating object to study in the future.

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