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CORRESPONDENCE

Sir,

Involvement of circulating endothelial progenitor cell and vasculogenic factors in the pathogenesis of diabetic retinopathy

The first point of their letter is that our study is not novel, and they suggest their studies confirmed the presence of abnormally high levels of circulating plasma markers of angiogenesis/vasculogenesis in patients with and without retinopathy. In their study, circulating plasma markers were confined to angiopoietin-1, angiopoietin-2, their soluble receptor Tie-2, vascular endothelial growth factor (VEGF) and its soluble receptor Flt-2, and they did not comment about the role of stem cells in the pathologic neovascularization. However, we tried to look at the mechanism of neovascularization from a different point of view in stem cell level, even though we could not conclude the exact mechanism of diabetic retinopathy.

We apologize for any confusion caused by the use of term serum instead of plasma in part of VEGF. Enzyme-linked immunosorbant assay samples were collected into ethylenediaminetetraacetic acid tubes with minimal stasis and centrifuged to obtain plasma. Indeed, VEGF was measured not in serum but in plasma. The correlation between the plasma level of VEGF and diabetic retinopathy were reported differently from many investigators. Shimada *et al*[1] found no association between the plasma VEGF level and retinopathy status in a larger number of type II diabetic patients (no retinopathy $n=30$, retinopathy $n=34$). We observed similar plasma level of VEGF in patients with and without established diabetic retinopathy.

We strongly agree that many associated co-morbidities (hypertension, cardiovascular disease, etc) and drug treatments (ACE inhibitors and statins) have to be fully taken into consideration in diabetic patients. As noted in our publication, we excluded patients from the beginning if they had a history of cardiovascular disease, administration of statin or ACE inhibitors, and human recombinant erythropoietin treatment.

To address the concerns of our study, it must be noted that 15 numbers of samples in each four groups were too small to elucidate complete statistical consequences. However, the findings that CD34+ cells and c-kit+ cells were elevated in diabetic retinopathy patients supports the hypothesis that neovascularization of diabetic retinopathy is a systemic vasculogenesis rather than a local angiogenesis. We believe that it is also likely that marrow-derived endothelial progenitor cells play an increasingly important role as a vasculogenic factors in diabetic retinopathy, although we acknowledge that more research and data to support this assertion are needed. We thank Dr KY Goon and YH Lip for having read and commented on our article.

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Sir,

Involvement of circulating endothelial progenitor cells and vasculogenic factors in the pathogenesis of diabetic retinopathy

In the May 2006 issue of *Eye,* Lee *et al*[1] present their findings from a small study which adds to our current understanding of pathological neovascularization in diabetic retinopathy. However, certain issues related to their study merit further discussion.

Their investigation of *systemic* (rather than local) angiogenic factors in diabetic retinopathy is not novel, as claimed by the authors. In 2000 and 2004, our research group reported plasma levels of vascular endothelial growth factor (VEGF), soluble VEGF receptor (Flt-1), angiopoietin-2 and its receptor tie-2 in patients with and without retinopathy.[2,3] Our studies confirmed the presence of abnormally high levels of circulating plasma markers of angiogenesis/vasculogenesis in these patients, suggesting an association between plasma indices of angiogenesis and the presence of diabetic retinopathy.

Of note, Lee *et al*[1] found no correlation between the severity of diabetic retinopathy and *serum* VEGF levels,

and cite the EUCLID substudy as corroborative evidence.[4] In that particular study, the researchers measured *plasma* as apposed to *serum* VEGF levels,[4] making a comparison difficult. Indeed, the use of serum to measure systemic VEGF levels has to be interpreted with caution, because activated platelets (during blood clotting) release VEGF into serum, and thus, results based on serum samples may be inaccurate in view of the possible artifact relating to the source of VEGF levels.[5] Also, the EUCLID study did report an upward trend in plasma VEGF levels, with increasing retinopathy (no retinopathy *vs* nonproliferative *vs* proliferative) (mean levels pg/ml: 11.5 *vs* 12.9 *vs* 16.1, respectively), an association that approached statistical significance ($P=0.06$) but was severely confounded by the disproportionate numbers in the groups (eg for nonproliferative $n=167$, proliferative $n=8$). In our own study, plasma levels of VEGF were elevated in diabetic patients, with the highest levels seen in more severe retinopathy (grade 2 and 3) ($P<0.05$).[3]

Furthermore, the title of their article[1] states circulating 'endothelial progenitor cells' but in fact, the study concentrates on stem cells (c-Kit+) and haematopoietic progenitor (CD34+) mononuclear cells. Increasingly, evidence is emerging that endothelial precursor cells are a heterogenous population with potentially different parent cell lines, but with some overriding phenotypic similarities in markers such as CD34, CD133, and VEGF receptor-2 (KDR). Furthermore, to prove endothelial lineage of these cells, uptake of acetylated low-density lipoprotein and Ulex Europaeus lectin should be demonstrated.[6] Thus, Lee *et al*[1] do not positively confirm that the increase in c-Kit+ and CD34+ cells in their study is due to a concurrent increase in the endothelial progenitor cell fraction, and we suggest that they should be more circumspect in their conclusions.

Lastly, the study by Lee *et al*[1] suffers from the obvious limitations of a cross-sectional design, and the many associated co-morbidities frequently seen with diabetic patients (hypertension, obesity, cardiovascular disease, etc) and drug treatments have to be fully taken into consideration, especially in a relatively small study. Many comorbidities in diabetics (as well as drugs such as angiotensin converting enzyme inhibitors and statins) could have a large effect on the various indices measured, making their results difficult to interpret.

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Sir,

Peripapillary choroidal neovascularization in Bietti crystalline retinopathy

Tapetoretinal degeneration with crystals at the corneal limbus, numerous yellow, glistening retinal deposits, and progressive atrophy of the retinal pigment epithelium (RPE) and choroid was reported by Bietti in 1937.[1] The corneal deposits are detected in only about one-third of patients, and when corneal changes are absent, the term 'Bietti crystalline retinopathy' is used.[2,3] The inheritance