Effects of Percutaneous Coronary Intervention on Peripheral Venous Blood Circulating Endothelial Cells and Plasma Indices of Endothelial Damage/Dysfunction*

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Background: **The relationship between endothelial damage/dysfunction and coronary artery disease is well recognized. However, the effects of percutaneous coronary intervention (PCI) [stenting/angioplasty] on circulating markers of endothelial damage/dysfunction (*eg*, von Willebrand factor [vWF], soluble E-selectin [sEsel] levels, and more recently circulating endothelial cells [CECs]) has been less well defined.**

Aims and methods: **We investigated the effects of both diagnostic coronary angiography (CA) [n = 15; blood sampling immediately before CA and 15 min after CA] and PCI (n = 38; blood sampling before PCI, 15 min after PCI, and 24 h after PCI) on levels of CECs, vWF, and sEsel across comparable patient groups. We also included a cohort of comparable healthy control subjects in order to compare baseline levels of three endothelial markers.**

Results: **There were no differences in baseline levels of CECs, vWF, or sEsel between the three study groups (healthy control subjects, CA, PCI; all p = not significant). Following CA (before to 15 min after), there were no significant changes in vWF and CECs (p = not significant). Following PCI, there were significant increases observed at 15 min after PCI and at 24 h after PCI (when compared with pre-PCI levels) in CECs (p = 0.0006), vWF (p = 0.007), and sEsel (p = 0.024).**

Conclusion: **We observed significant increases in three endothelial markers (CECs, vWF, and sEsel) with elective PCI but not CA. This is in keeping with endothelial damage/dysfunction following PCI.** ***(CHEST 2007; 132:1920–1926)***

Key words: angioplasty; circulating endothelial cells; coronary; endothelial; soluble E selectin; stenting; von Willebrand factor

Abbreviations: CA = coronary angiography; CAD = coronary artery disease; CEC = circulating endothelial cell; CI = confidence interval; PCI = percutaneous coronary intervention; sEsel = soluble E-selectin; vWF = von Willebrand factor

Circulating endothelial cells (CECs) are a novel and direct marker of endothelial injury and dysfunction, and as such represent a major growth area in experimental research.[1,2] CECs are endothelial cells that have become detached from the endothelial wall and released into the circulation in response to endothelial damage.[1] Increased CECs have been identified across a broad spectrum of cardiovascular disease states[1] and have been linked to the clinical severity of coronary artery disease (CAD), as well as to several adverse clinical end points.[1–4] Of note, there have been several recent technical advances in CEC isolation methodology, allowing improved data validation.[1,5,6] Also, a consistent relationship has been demonstrated between CECs and well-established markers of endothelial injury/damage, including soluble E selectin (sEsel), tissue factor, and von Willebrand factor (vWF).[3,7–9]

Previous work,[10,11] including some from our group,[12] have consistently suggested that percutaneous coronary intervention (PCI) [angioplasty with or without stenting] leads to a significant increase in venous blood CEC

counts (> 50%) compared with preprocedural levels. However, the source of these cells (that is, coronary vs systemic) remains unclear, especially since passage of cardiac catheters and/or guide wires could potentially cause endothelial perturbation in peripheral arteries or the aorta. Secondly, the factors influencing their release and relationship between CECs and other levels of other markers of endothelial dysfunction following PCI have been poorly defined.

In this study, we hypothesized that both routine coronary angiography (CA) and PCI would lead to a significant increase in CECs but with a greater increase following PCI, compared with CA. Secondly, we hypothesized that there would be a positive relationship between increasing CEC counts and levels of vWF and sEsel, in keeping with their shared endothelial origin.

Materials and Methods

We performed an observational study that included three patient groups aged 35 to 80 years. The first patient group consisted of patients scheduled for elective PCI for stable CAD. The second but smaller patient group consisted of patients scheduled for routine CA. The purpose of the latter group was to ascertain the effects of diagnostic CA on CEC counts and other markers of endothelial damage. This group consisted of patients with symptoms consistent with stable CAD, and listed for elective diagnostic CA.

We excluded patients with any of the following: a history of liver disease; dialysis or with a serum creatinine level > 200 μmol/L; malignancy; recent (< 3 months) arterial or venous thromboembolic disease or myocardial infarction; active infections and/or a history of inflammatory or connective tissue disorders; and uncontrolled BP > 180/110 mm Hg. Patients undergoing PCI and CA were compared to a third study group, which consisted of healthy control subjects of comparable age, sex, body mass index, and ethnicity. Healthy control subjects were identified by a detailed history and physical examination, with a normal baseline full blood count, renal function, fasting glucose, and lipid profile. Healthy control subjects consisted of healthy volunteers responding to local advertisement, relatives of known patients, and local members of staff. The rational for the inclusion of a healthy control group was in order to give a perspective of levels of CECs, vWF, and sEsel in health compared with "diseased" patient groups, rather than to emphasize the cases vs "healthy control" comparison. All patients underwent written and informed consent prior to inclusion into the study. The study was fully approved by the West Birmingham Research Ethics Committee.

Sampling Techniques

All diagnostic coronary angiograms was performed using 5F and 6F sheaths (Cordis; Warren, NJ) via the right femoral and radial arteries, respectively. All PCI procedures were performed using 6F sheaths via right femoral and radial arteries. The extent of angiographic CAD was estimated using the calculated Gensini scoring system.[13] All patients in the PCI group were receiving dual-antiplatelet therapy with aspirin and clopidogrel prior to the procedure.

Sampling Schedule

All blood samples were obtained from venous sites from the arm. Preprocedural blood samples were obtained within 1 h of the procedure from supine rested patients. Post-PCI and angiogram blood samples were obtained at 15 min after the procedure. For the PCI groups, a third blood sample was obtained at 24 h after the procedure. At least the first 4 mL of aspirated blood was discarded at every venepuncture. This was undertaken in order to reduce any potential influence of the needle passage through the endothelial wall on the release of endothelial markers.[14] The operator was blinded to the sample order. All second blood samples were obtained prior to removal of the femoral sheath or use of a vascular closure device (Angioseal; St. Jude Medical; Minnetonka, MN).

Analysis of Endothelial Markers

We used the immunobead technique of CEC isolation (using CD146-coated immunomagnetic beads) with cellular counter staining using fluoroscein isothiocyanate-stained endothelial-specific *Ulex europeus* lectin. Our detailed method (including assay variability) has been both well described and validated.[5,6,14] All venous blood samples for CEC quantification were transported at room temperature. CECs were defined, on fluorescent microscopy, as cells 10 to 50 μm in size with four or more immunobeads attached and staining positive for fluoroscein isothiocyanate-stained *U europeus*.

For vWF and sEsel determination, all samples were collected, transported on ice, and separated by centrifugation at 3,000 revolutions per minute (1,000*g*) for 20 min at 4°C to obtain citrated plasma (for vWF) and serum (sEsel), which was then stored at − 70°C to allow later batch analysis. vWF levels were measured in duplicate by enzyme-linked immunosorbent assay using commercial reagents (Dako-Patts; Ely, United Kingdom). sEsel was measured by enzyme-linked immunosorbent assay with reagents (R&D Systems; Abingdon, UK). Intraassay and interassay coefficients of variation for vWF and sEsel were and < 5% and 10% and < 5% and < 12%, respectively; lower limits of detection were 0.5 IU/dL and 0.5 ng/mL, respectively.

Power Calculation and Data Analysis

Power calculations were performed using software (GraphPad StatMate version 2.00 for Windows; GraphPad Software; San Diego CA; www.graphpad.com). Previous work by ourselves (n = 26)[12] and others (n = 10 to 15)[10,11] have demonstrated a > 50% increase in venous blood CECs following PCI. Based on these data—and given that CECs were the primary research

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