Supplemental File 1: Endothelial damage and dysfunction methodology

Assessment of CD31+/AnnexinV+/CD42b- EMPs

Peripheral venous blood was collected from the study participant in a 5ml citrated vacutainer using a 21g needle. Platelet-poor plasma (PPP) was generated using a two-step centrifugation process. The venous sample was initially centrifuged at 1700g for 10 minutes at 4°C to generate plasma, which was harvested and centrifuged at 20,000g for a further 10 minutes at 4°C. The PPP was harvested and the final sample frozen in aliquots at -80°C to analyse in batches. We added 50µl of PPP and 50µl of 10µm diameter counting beads (Flow-Count™ Fluorospheres; Beckman Coulter, UK) to 900µl of calcium-rich buffer (Annexin V Apoptosis Detection Kit, eBioscience, UK). Simultaneous incubation for 10-minutes with fluorescent antibodies was performed using 5µl of phycoerythrin (PE)-conjugated antihuman CD31 (BD Pharminogen 55546), 5µl of allophycocyanin (APC)-conjugated antihuman CD42b (BD Pharminogen 551061), and 10µl efluor450 annexin-V marker (Annexin V Apoptosis Detection Kit eBioscience, UK). Flow cytometry was performed on prepared samples using the Beckman Coulter Cyan ADP flow-cytometer with Summit V4.3 software. Analysis was stopped once 1000 beads had been counted and gates were set to exclude artefact and beads. Events negative for CD42b (platelet-marker) were selected and events positive for CD31 (endothelial marker) and Annexin V (microparticle marker) were defined as CD31+/AnnexinV+/CD42b- EMPs. Absolute EMP counts per millilitre of plasma were then calculated.

Assessment of endothelial function (FMD (%))

All scans were performed on fasting subjects between 08:00 and 10:00, and vasoactive medications were withheld for 24 hours. Participants were examined in a temperature-controlled room (21-24°C), after a period of rest and equilibration. Endothelial-dependent FMD of the brachial artery was assessed using B mode ultrasound and a 12 MHz probe (Philips ATL HDI 5000) linked to a personal
computer (PC) via a BNC cable (National Instruments). The PC was equipped with a frame grabber (National Instruments PCI 1405), vision deployment engine (National Instruments) and automated edge-tracking software (Vascular Image Analysis (VIA), MD Medics). The brachial artery was identified longitudinally, 5-10cm proximal to the ante-cubital fossa, and the probe was fixed in position using a stereotactic probe-holder. Depth and gain adjustments were made to obtain the best possible wall-lumen interface and a reference image was saved to disk, and referred to on the 2nd visit. A region-of-interest was selected and the walls of the brachial artery automatically tracked for the entirety of the test. B-mode ultrasound images were processed at 25 frames per second and the vessel diameter was displayed in real time. Baseline measures were recorded for at least 2 minutes and the BP cuff was then inflated to 50mmHg above the resting systolic blood pressure (to at least 200mmHg) for 5 minutes. Following cuff deflation, Doppler ultrasound was used to confirm reactive hyperaemia and recording continued until the arterial diameter had returned to baseline. Endothelial-independent dilatation of the brachial artery was then assessed following the administration of sublingual glycercyl trinitrate (GTN) 300mcg. Automated on-line analysis was performed immediately and average baseline brachial artery diameter (mm), average diameter at 60 seconds post-deflation (mm), and FMD (%) at 60 seconds were automatically calculated by VIA. Peak brachial artery diameter and peak FMD were also manually selected and recorded if different to the pre-set 60-second time point. Percentage FMD was calculated as follows:

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\text{% FMD} = \left(\frac{\text{mean peak diameter} - \text{mean baseline diameter}}{\text{mean baseline diameter}}\right) \times 100\%
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In a preliminary study to validate the technique and protocol, the correlation coefficient for paired measures of baseline brachial artery diameter and FMD (%) in 8 healthy subjects at least 1 week apart was 0.95 (p = 0.0003) and 0.79 (p = 0.02), respectively.