

AN INCREASE IN CIRCULATING MONOCYTE- AND PLATELET-DERIVED MICROPARTICLES DURING HAEMODIALYSIS

Naomi Martin^{1,2}, Maurice Dungey^{1,2}, JO Burton² Hannah ML Young², Alice C Smith^{1,2}, Nicolette C Bishop^{1,2}

¹School of Sport, Exercise and Health Sciences, Loughborough University, UK

²Leicester Kidney Exercise Team, University of Leicester and University Hospitals of Leicester, UK

Haemodialysis (HD) patients have a dysfunctional and chronically activated immune system and are at very high risk of CVD. Microparticles (MP) are biologically active nanovesicles shed from cells into the vasculature during activation, are a novel biomarker of systemic inflammation and are associated with increased risk of CVD. Circulating numbers of MP are reported to be higher in HD patients, but the source and potential function of these is unknown. This study assessed the acute effect of HD on the total number, cellular origin and percentage of pro-thrombotic MP. The functional ability of these MP to induce endothelial cell (EC) reactive oxygen species (ROS) *in vitro* was also investigated.

11 patients (mean±SD ; age 57.6±9.4 yr; 7 Male; HD vintage 40±33 months) were studied during a routine HD session. Blood samples were taken directly from the HD access 60' after HD commenced (to allow for stabilization) and at 100', 160' and 240' during HD. MP were identified flow cytometrically using MegaMix beads, and enumerated using FlowCount beads. The numbers and proportions of pro-thrombotic MP expressing Phosphatidylserine (PS) and tissue factor (TF) were assessed. The cellular origin of the MP was identified by phenotypic staining (EC, monocyte (Mo), neutrophil (PMN) and platelet (PLT)). In an *in vitro* co-culture of MP with a human endothelial cell line (EAHy), the ability of the HD-derived MP to induce ROS was quantitated using the fluorimetric probe dichloro-dihydrofluorescein diacetate (DCFH-DA).

A significant increase in total MP numbers was observed over the course of HD (mean±SD; 234±270 to 1049±973 x10⁶/μl; *P*=0.008). Numbers of both PLT- and Mo-derived MP significantly increased during HD (7.81±6.96 to 19.54±11.71 x10⁶/μl; *P*=0.003 and 5.11±6.45 to 12.46±13.39 x10⁶/μl; *P*=0.02). The percentage of PMN-MP decreased (3.59±5.42 to 1.49±2.48 %; *P*=0.03). MP derived from EC or expressing TF did not change.

In contrast, the percentage of prothrombotic PS MP decreased during HD (5.02 ± 4.70 to 2.27 ± 1.92 %; $P=0.02$). When MP collected over the course of HD were incubated with cultured EC overnight, a decrease in ROS production was observed (0.19 ± 0.04 to 0.03 ± 0.01 DCFHDA signal/ 10^6 MP; $P=0.000$).

In agreement with previous reports, an increase in MP numbers was observed during HD. However, this is the first study to demonstrate differential changes in the cellular origin of these MP, whereby substantial increases in PLT-MP and Mo-MP were observed, yet proportions of PS-expressing MP and those derived from PMN decreased. Furthermore, the functional ability of these MP to induce EC ROS production was diminished during HD. These novel findings add further insight into the intravascular inflammatory consequences of HD which may contribute to the extreme vulnerability of this population.