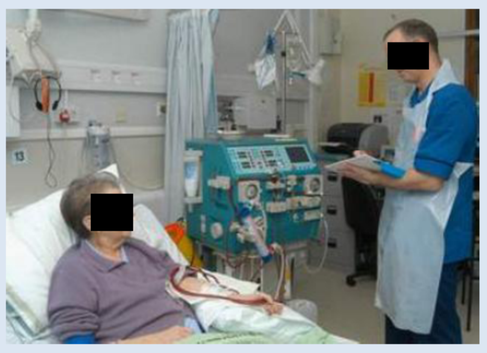
Leicester Kidney Exercise Team

An increase in circulating monocyte- and platelet-derived microparticles during haemodialysis.

Martin N[1,2], Dungey MR[1,2], Burton JO[1,2], Young HML[2], Smith AC[1,2], Bishop NC[1,2]
[1]School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UNITED KINGDOM
[2]Leicester Kidney Exercise Team, Department of Infection, Immunity & Inflammation and John Walls Renal Unit, Leicester General Hospital, Leicester, UNITED KINGDOM

Introduction

- Haemodialysis (HD) patients have a dysfunctional immune system that is paradoxically both chronically activated yet anergic, associated with an increased risk of atherosclerosis and cardiovascular disease (CVD).
- Microparticles (MP) are biologically active nanovesicles shed from activated systemic cells into the circulation. MP are a novel biomarker of systemic inflammation and are associated with increased risk of thrombosis, CVD and disease pathology.
- Circulating MP have been shown to be increased in number in HD, and MP derived from endothelial cells (EC), neutrophils (PMN) and platelets (PLT) are increased in HD patients.
- Previous work investigating changes in MP before and after HD have identified HD-induced increases in PLT- and PMN-derived and pro-thrombotic MP, but these studies have largely assessed MP before and after HD, not during the HD procedure itself.



Aims

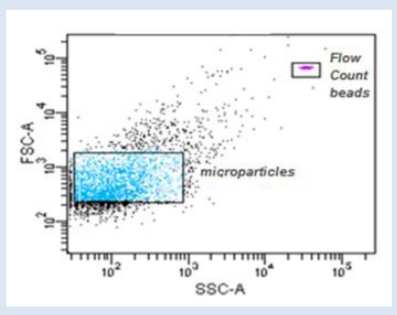
To assess the effect of haemodialysis on:
- The number of circulating MP
- The number of pro-thrombotic MP
- MP cellular origin
- The ability of these MP to induce EC ROS

Methods

Patients
- 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 min (to allow for stabilization), 100 min and 160 min after HD commenced, and at the end of the HD session.
- MP were isolated by serial centrifugation and washed with filtered HEPES buffered salt solution.

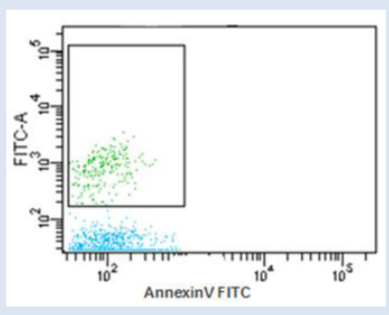
Flow Cytometric Analysis
- Aliquots of MP were incubated alone, with AnnexinV-fluorescein isothiocyanate (AnV+, to identify surface phosphatidylserine (PS)), or with anti-human phycoerythrin-labelled antibodies to determine cellular origin, or appropriate isotype control antibodies
- MP were enumerated using Flow-Count Fluorospheres™. All flow cytometric acquisition was done using a FACSCanto II. MP were acquired using gating defined by size-calibrated fluorescent Megamix™ beads.
- The numbers and proportions of pro-thrombotic MP expressing PS and phenotype of the MP was identified using antibodies against the following cellular markers: PLT CD42b, EC CD144, tissue factor (TF) CD142, PMN CD66b, monocyte (Mo) CD14.

Representative flow cytometric plots:

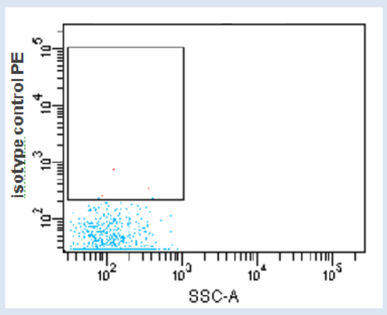


MP enumeration

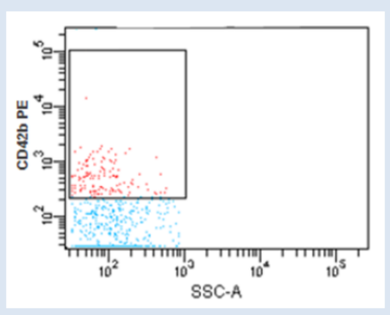
FACSCanto II flow cytometer

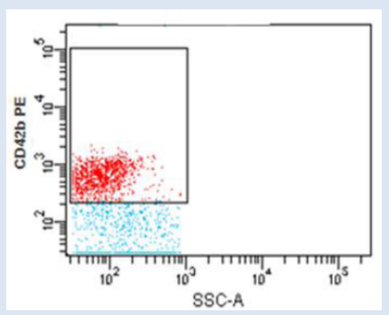


Identification of AnV+ PS MP



Isotype control gating





Identification of platelet-derived MP 60 min into & at the end of HD

Microparticle-induced reactive oxygen species in an endothelial cell line
- EA.hy926 endothelial cells were seeded onto 96-well plates and loaded with dichlorodifluorescein diacetate (DCFH-DA) prior to the addition of MP (prepared and enumerated as above).
- Fluorescence at 529nm as a measure of ROS was measured every 15 min for 2 hrs, and then at 20 hrs with incubation in a humidified 5% CO_2 atmosphere at 37ºC using a Varioskan ™ Flash Multimode Reader.
- Each treatment was carried out in duplicate, n=6. Results are given as DCFH-DA signal per 10^6 MP, minus background and buffer readings.

Results

- **There was a significant increase in total MP numbers over the course of HD** (mean±SD; 234±270 to 1049±973 x10^6/µl; *P*=0.008) (Figure 1).
- **The percentage of pro-thrombotic AnV+ MP decreased over the course of HD** (5.02±4.70 to 2.27±1.92 %; *P*=0.02) (Figure 2).

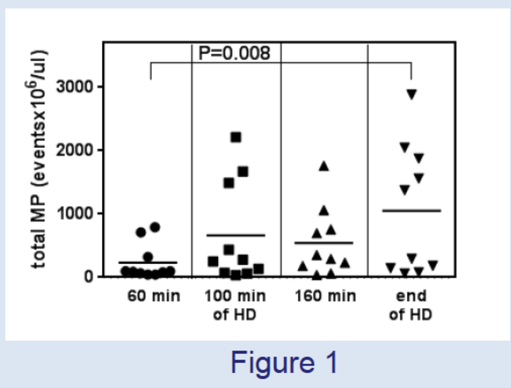


Figure 1

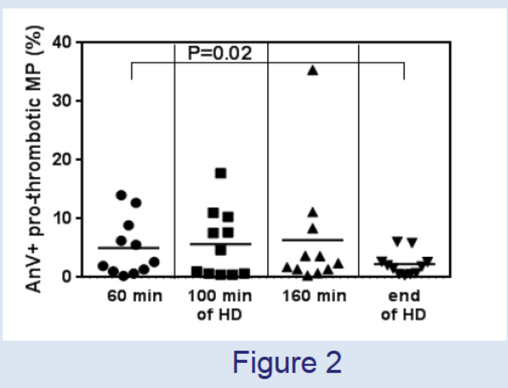


Figure 2

- **HD induced changes in the cellular origin of MP.**
  - Numbers of PLT-derived MP significantly increased during HD (7.81±6.96 to 19.54±11.71 x10^6/µl; *P*=0.003 (Figure 3).
  - Mo-derived MP increased significantly during HD (5.11±6.45 to 12.46±13.39 x10^6/µl; *P*=0.02) (Figure 4).
  - The percentage of PMN-MP decreased (3.59±5.42 to 1.49±2.48 %; *P*=0.03) (data not shown).
  - MP derived from EC or expressing TF did not change (data not shown).

- 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*<0.001) (Figure 5).

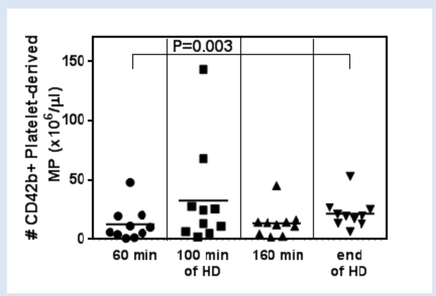


Figure 3

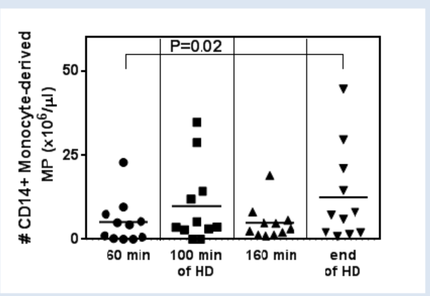


Figure 4

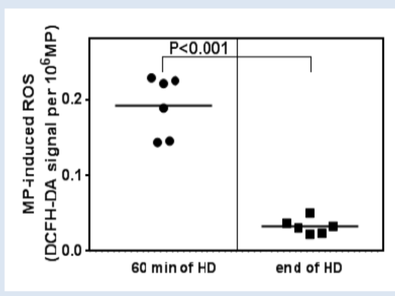


Figure 5

Conclusions

- In agreement with previous reports, an increase in total MP numbers was observed during HD.
- However, this is the first study to demonstrate comprehensive differential changes in the cellular origin of these MP during the HD session.
- Substantial increases in PLT-MP and Mo-MP were observed, yet proportions of PS-expressing pro-thrombotic 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.

Dr Naomi Martin: n.martin@lboro.ac.uk

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