**Title:** Determining the role of platelet-red blood cell aggregation in severe diabetes related foot disease

**Abstract:**

Background: In patients with severe diabetic foot disease (DFD) there are increased levels of morbidity and mortality, and high associated healthcare costs. We hypothesise that tissue sepsis, ischaemia and necrosis associated with severe DFD lesions result in release of histones from nuclear chromatin into the circulation. This can result in platelet (P)/red blood cell (RBC) (P-RBC) aggregation, endothelial injury and a systemic inflammatory response. P-RBC aggregates can increase thrombosis risk.

Aim: To ascertain if P-RBC aggregates are increased in patients with diabetes with severe DFD compared to without DFD.

Methods: In single-centre pilot case-control study (November 2024-April 2025) we assessed baseline clinical and pathology characteristics of five patients with severe DFD requiring surgical intervention and six age and sex matched controls with diabetes but without DFD. P-RBC aggregates were assessed by flow cytometry and expressed as % of RBCs.

Results: The cases with severe DFD were well matched with controls for age (Mean±SD 62±9 vs 65.2 years, p=0.69), sex (60% vs 50% female), and mean duration of diabetes (20±9 vs 18±11 years, p=0.96). All cases had history of peripheral neuropathy, with 80% having history of prior foot ulceration and 60% with history of prior amputation for DFD. The mean white blood cell count was elevated in cases vs controls (11.2±2.8 vs 7.5±0.9 x109per L, p=0.01), whereas no differences were found in haemoglobin concentration or platelet count. Acute phase reaction markers were higher in cases vs controls (fibrinogen 5.6±1.9g vs 3.7±1.0 g/L, p=0.04; and CRP 95.4±64 vs 5.5±5.9 mg/L, p < 0.01). Mean P-RBC aggregate were 57% higher in cases vs controls (0.58±0.14 vs 0.33±0.01 %, p=0.025).

Conclusion: In this pilot study, we demonstrated that P-RBC aggregates were increased in patients with severe DFD compared with diabetic controls without DFD.