

## Non-classical monocytes increase after fistuloplasty of an arteriovenous fistula and may promote restenosis

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**Introduction:** An arteriovenous fistula (AVF) is the preferred method for providing vascular access for haemodialysis. Stenotic lesions caused by neointimal hyperplasia (NIH) commonly occurs in fistulas resulting in patients requiring a fistuloplasty procedure, yet restenosis in the AVF occurs in nearly half of patients within their first year of their procedure by poorly defined mechanisms. The purpose of this study is to investigate the factors which may lead to restenosis occurring in the AVF following a fistuloplasty procedure.

**Methods:** Resected AVF tissue from 4 patients were histologically characterised. The expression of 50 proteins were analysed in 30 pairs of plasma samples taken pre- and 1-day post-fistuloplasty via Luminex and ELISAs. These samples were from patients recruited to the PAVE trial but then found to be ineligible and not randomised. Using flow cytometry, lymphocytes and monocyte subsets were analysed in 20 pairs of pre- and post-fistuloplasty cryopreserved peripheral blood mononuclear cells (PBMCs). Monocyte populations were further investigated by carrying out flow cytometry on fresh whole blood with counting beads from 5 patients undergoing a fistuloplasty. This allowed assessment of absolute cell numbers.

**Results:** Histological findings revealed that most of cells within the neointimal lesion are myofibroblasts (alpha SMA+), with smaller numbers of contractile (SM-MHC+) smooth muscle cells. AVF tissue from different patients had variations in the number of CD68+ and CD34+ cells within their neointimal lesions. Plasma myeloperoxidase significantly decreased 1-2 days after the fistuloplasty procedure, whilst IL-6 and TNF-a significantly increased. The proportion of intermediate (CD14++CD16+) and non-classical (CD14+CD16+) monocytes increased one day post procedure. Time course experiment in 5 patients with fresh whole blood confirmed the increase in the proportion of non-classical monocytes at day 1. They further showed no change at 3 hours and demonstrated that the proportional changes were due to an increase in the number of non-classical monocytes and not a decrease in classical monocytes. There were no changes in CD4+ T cells, CD8+ cells, gamma delta T cells, CD19+, or NK cells.

**Discussion:** The phenotype of CD16+ non-classical monocytes released post-fistuloplasty requires further study. The presence of CD68+ monocyte-like cells within the neointima suggests that CD16+ cells may infiltrate the neointima and have pathogenic potential. Furthermore, myeloperoxidase, IL-6 and TNF-a may be of importance at the local site of injury during restenosis.

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