

# Fifteen-year follow-up of patients with critical limb ischaemia after local cellular therapy: the long-term positive effect is due to the presence of CD45<sup>+</sup> CD34<sup>+</sup> and CD34<sup>+</sup> cells in the inoculum

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## Introduction

Bone marrow constitutes a reservoir of progenitor cells which, being at different stages of differentiation, stay dormant until mobilised by signals coming from outside. It was postulated in the early 2000s that endothelial progenitor cells may leave the marrow under the force exerted by the hypoxia gradient to settle in ischaemic tissues. With that in mind, in 2003 we started a project of revascularisation of the limbs of patients suffering from non-option critical limb ischaemia. Since then, two cohorts have been enrolled, the first experimental (2003–2005, 13 patients, 16 cellular therapies (CT), two female and 11 male, age 32–64 years), and the second (2009–2011, 16 patients, 16 CT, four female and 12 male, age 34–64 years) to validate the primary cohort results.

## Methods

On the day of the procedure, (i) the bone marrow was harvested from the posterior iliac crest in a volume of 500 mL, (ii) the cells were enriched in mononuclear cells using a Cobe Spectra separator; the obtained leukophoretic product (LP) was about 100 mL in volume (CT product), (iii) 70 mL of the cell suspension was injected in 0.7 mL portions into calf muscles of the affected limb.

## Results and discussion

Outcome of the procedure: cohort 1 and (cohort 2) benefited with: pain reduction in 83% (88%), 67% (57%) and 28% (20%) of cases, and wound healing in 25% (38%), 42% (43%) and 50% (33%) of cases, when assessed 1, 3 and 12 months after CT, respectively. The similar outcome seen in the two cohorts led us to perform further analysis in both cohorts together. The follow-up of patients revealed that a long-lasting effect was present at 4 years after CT in more than 50% of patients.

In three patients, the CT was repeated with a beneficial effect. In six cases, amputation was needed between 3 and 9 years after therapy. Four patients died due to atheromatous lesions, but in two, limb ischaemia symptoms were still absent. Median observation time of the first cohort group was 14 years.

LP was enriched compared with the marrow (t-test for paired samples) in: CD45<sup>+</sup> CD34<sup>+</sup> (0.05 vs 0.02%,  $p < 0.046$ ), CD73<sup>+</sup> CD34<sup>+</sup> CD45<sup>+</sup> (0.07 vs 0.04%,  $p < 0.002$ ), CD31<sup>+</sup> CD34<sup>+</sup> CD45<sup>+</sup> (0.08 vs 0.03%,  $p < 0.05$ ).

A positive response seen 1 month after CT (the population of cells was analysed for clinical symptoms) was associated with a high proportion of CD34<sup>+</sup> cells in LP (median: 1.53 vs 1.19%,  $p < 0.03$ ), but not with higher proportions of CD45<sup>+</sup> CD34<sup>+</sup> CD90<sup>+</sup> and CD73<sup>+</sup> cells compared with the failure group.

Muscles biopsied on the day of CT had significantly higher expression of the *SDF-1* gene but lower of *CXCR4* compared with the marrow cells.

## Conclusions

Bone marrow cells enriched in mononuclear cells with an endothelial progenitor phenotype are effective in non-option patients with critical limb ischaemia; unwanted effects were absent, and this observation is especially of note as nine cases were observed for longer than 10 years; the social effect was impressive due to improvement in the quality of life, restoring normal social activity and cessation of painkillers including narcotics. ■

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