Materials and methods

Fourteen adult female C57Black6 mice were immunized with methylated bovine serum albumin (mBSA). Arthritis was induced by a single intra-articular injection of 60 µg mBSA. To visualize perfusion of vessels, the animals were injected intravenously via one of the tail veins with a 0.2 ml solution of phosphate-buffered saline (PBS, pH 7.4) containing 0.3 mg Hoechst 33342 (Sigma Chemical Company, St.Louis, MO), on days four and day seven after induction of arthritis. Hoechst is a fluorescent dye that is rapidly taken up by the nucleus of endothelial cells. Exactly one minute after Hoechst injection the mice were killed and both knees were quickly embedded in Tissue Tek, preventing the Hoechst dye to diffuse further into the tissues. Thereafter the knee joints were sectioned (7 um) without previous decalcification, at -22°C. Sections were stained with a monoclonal rat-anti-mouse endothelial cell marker (9F1) and with goat-anti-rat-TRITC as secondary antibody.

Immediately after the staining procedure the localization of Hoechst and the vascular marker 9F1 were analyzed with an automated digital image processing system. This system was programmed to measure synovial area (SA), vessel area (VA), number of perfused vessels (pVA), number of blood vessels (NBV), number of perfused blood vessels (NpBV), and the (perfused) vessel density (N(p)BV/SA=(p)VD). Data were analyzed with the Wilcoxon signed rank test for the comparison between the left control knee and the right arthritic knee of each mice and for the differences between arthritis day four and seven more (perfused) vessels were present as compared to arthritis day four and seven had increased significantly compared to the controls, and on day seven more (perfused) vessels were present as compared to day four (Fig. 1).

Discussion

The image analysis system allows analysis of whole sections of the mouse knee joint. The main advantage of this method is that both perfused and the total number of vessels can be measured in the same section quantitatively. This makes this procedure very efficient for quantitation of the effects of the different forms of drug therapy that have blood vessels as their target. **Dept Rheumatology, ***Dept Radiotherapy, University of Nijmegen.