Methods

Design Overview

This prospective non-randomized interventional pilot study was conducted in 1 French centre from April 2013 to June 2015 in the Dermatology Department of Caen University Hospital (France). It was carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from each patient before enrolment in the study. The local Ethics Committee and Regulatory Authorities approved the study protocol.

Setting and Participants

Inclusion criteria were patients aged 18 years or older, ability to give informed consent and follow the treatment procedure and presence of 1 HLU of 1–300 cm^2 in total area. HLU was defined by clinical criteria: superficial and painful skin necrosis surrounded by a characteristic purpuric margin, rapidly spreading, located on the lower part of the leg. All patients had adapted antihypertensive medication. For all preselected patients, the initial examination revealed no signs of chronic arterial or venous insufficiency of the lower extremities, and peripheral pulses were palpable (Fig. 1). Every patient had previously undergone an immunological laboratory blood test, blood glucose test, arterial-venous Doppler ultrasound of the lower limbs and a 4-mm punch skin biopsy from the ulcer wound margin. Exclusion criteria were cutaneous vasculitis, peripheral arterial occlusive disease – ankle brachial disease index of 0.8 or less – or severe chronic venous insufficiency, cryoglobulinaemia, thrombophilia, evolving systemic or other autoimmune disease, prior or evolving cancer, haemopathy or concomitant treatment with corticosteroids, immunosuppressive, cytotoxic drugs or hydroxyurea treatment. Minor subjects, pregnant women, and persons deprived of liberty or unable to give their consent were not included.

Intervention: Lipostructure®

Micro-reinjection of autologous fat was performed in the Department of Maxillofacial and Plastic Surgery, under general anaesthesia. Adipose tissue donor sites were located in the medial area of the knee and the abdominal and trochanteric regions. The Lipostructure® was codified in 3 steps by Coleman in 1994 [16–19]. It can be performed with specific smaller-calibre cannulas developed by G. Magalon, called micro-reinjection of autologous fat [20]. Firstly, adipose tissue is harvested without any incision, using a specific 2-mm cannula. Secondly, the harvested fat tissue is centrifuged at 2,000–3,000 rpm to separate the tissue from its water content and from the oil produced by the destruction of damaged adipocytes. The upper level is the least dense and consists primarily of oil, the middle portion is primarily fatty tissue, and the lowest layer is blood, water, and lidocaine. Thirdly, purified fat (middle portion) is reinjected with specific 0.8-mm cannulas. Around and under the wound, deposits of micrografts of fat are positioned in multiple layers localized in the subdermal stratum (Fig. 2). This technique, more precise because the fat deposits are thinner, preserves the histological structure and ensures a better viability of the cells in the subdermal stratum [20, 21].

Outcomes and Measurements

The primary outcome was the wound closure rate after Lipostructure®, defined as the percentage reduction in the original wound surface area (surface area at time t – surface area at D0/surface area at D0). The secondary outcomes were the wound characteristics (necrosis, fibrin, granulation tissue), pain assessed with a visual analogue scale (range 0–10 cm), and adverse events. Clinical evaluations were performed by 2 investigators (M.C., A.D.) before treatment, on the day when Lipostructure® was carried out (D0), 7 days later (D7), and every month thereafter for 6 months (M1, M2, M3, M4, M5, and M6). At each evaluation, photographs were taken in optimal lighting conditions with a 10-cm ruler. Images were automatically converted to the JPEG format by the camera with a resolution of 3,264 × 2,448 pixels and then transferred to a compatible computer. An independent expert nurse (B.M.) using a mouse on photographic images manually delineated surfaces of yellow fibrin, black necrosis and red granulation tissue. The surfaces and percentages of the various wound constituents were measured using a computerized planimetry software package, Canvas (ACD Systems, BC, Canada), already evaluated in previous studies [22].

Statistical Analysis

The medians and quartiles of the wound closure rates were calculated at each visit. A non-parametric bootstrap resampling technique (bias-corrected and accelerated method, 2,000 samples) was used to estimate the 95% bilateral confidence intervals of the medians and to evaluate the significance of the decrease in the wound surface areas (confidence intervals excluding 0), compared to D0. The wound closure rates were compared over time with a non-parametric Friedman test. The median and quartiles of each of the secondary end points (necrosis, fibrin, and granulation tissue percentages, pain score) were calculated at each visit and compared over time with non-parametric Friedman tests. The significance level was set at $p < 0.05$. All analyses were conducted using IBM SPSS software (version 22).