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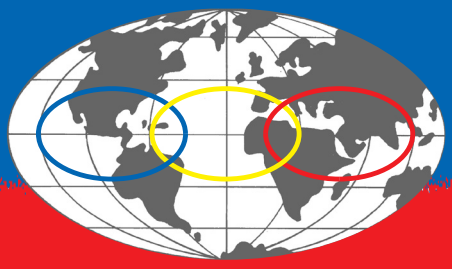

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Health-related quality of life outcomes and hospitalization length of stay after micro-fragmented autologous adipose tissue injection in minor amputations for diabetic foot ulceration (MiFrAADiF Trial): results from a randomized controlled single-center clinical trial

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ORIGINAL ARTICLE

Health-related quality of life outcomes and hospitalization length of stay after micro-fragmented autologous adipose tissue injection in minor amputations for diabetic foot ulceration (MiFrAADiF Trial): results from a randomized controlled single-center clinical trial

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ABSTRACT

Background: The diabetic foot ulcer (DFU) is a high prevalence complication that significantly impairs the health-related quality of life (HRQoL) and is characterized by prolonged hospital length of stay (LOS). The impact of the micro-fragmented autologous adipose tissue injection at the minor amputation wound in the case of DFU (MiFrAADiF) on HRQoL and LOS compared to the standard care has not been determined yet.

Methods: This was a two-arm, 6-month, individually-randomized controlled single-center clinical trial. A 1:1 randomization to local injection of autologous micro-fragmented adipose tissue (treatment group; N.=57) or standard clinical care (control group; N.=57) was performed. The primary objective was the HRQoL. The secondary endpoint was the LOS. HRQoL was assessed with the Medical Outcomes Study 36-item Short-Form Health Survey which provides 2 scores focused on physical (PCS) and mental functioning (MCS). The trial was registered in ClinicalTrials.gov (NCT03276312).

Results: The type of treatment (P=0.009) and the time elapsed since surgery (P=0.0000) demonstrated a significant improvement on PCS. The MCS improvements resulted in a non-significant association with treatment (P=0.21). The time elapsed since surgery showed a significant influence on the MCS (P=0.0000). The mean LOS was 16.2 days and 24.4 days for the treatment and the control group respectively (P=0.025).

Conclusions: The MiFrAADiF Trial demonstrated a significant improvement in terms of physical HRQoL and a significant reduction of the hospital length of stay after injection of micro-fragmented autologous adipose tissue in diabetic patients' minor amputations wound.

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Key words: Amputation; Diabetes complications; Foot ulcer; Surgical procedures, operative; Stem cells; Adipose tissue.

Diabetes mellitus is recognized as a high prevalence disease affecting more than 600 million diabetic patients within 15-years as reported by the International Diabetes Foundation. Diabetes-related foot complications have been increasing over the last decades with a global prevalence of 6.3%.[1-5] According to the Eurodiale Study,[6] up to 50% of diabetic foot ulcers (DFU) were diagnosed with concomitant peripheral arterial disease and infection representing a complex clinical scenario. Such as other heavily diseased conditions, the DFU presented a relevant increase of amputation risk.[7, 8] Besides, the presence of DFU decreased significantly the health-related quality of life (HRQoL).[9] In case of DFU, a multidisciplinary approach should be undertaken providing general health advices (including adequate shoes), evaluating glycemic control, excluding vascular and infective issues, and performing adequate dressings.[2-4] As stated, DFU suffers a significant failure of the conservative approach evolving in lower extremities amputation. A "minor" amputation (digital or transmetatarsal) is able to preserve the foot load and does not influence negatively the HRQoL when compared with conservative treatment.[10] Therefore, it should be preferred when feasible. However, 30% of transmetatarsal interventions require subsequently an ipsilateral major amputation.[11] It seems reasonable to improve healing of minor amputations and to preserve foot viability. Stem cell-based therapies have emerged as a very interesting therapeutic strategy to improve the healing process.[12-14] The adipose tissue mesenchymal stem cells are numerous, easy to access and demonstrated regenerative properties and favorable initial results when applied in DFU.[14, 15] The first randomized trial regarding the micro-fragmented autologous adipose tissue injection in minor amputations wound for diabetic foot ulceration (MiFrAADiF) was recently published by our group.[16] We demonstrated a significant benefit in terms of healing when compared to standard care.[16] Aiming to reply to other authors claiming "evidence-based interventions" to improve HRQoL outcomes in patients with DFU, we reported these outcomes coming from the MiFrAADiF trial.[9]

To the best of the authors' knowledge, there are no publications focused on wound healing impact on HRQoL outcomes (physical and mental health) and hospital length of stay (LOS) after autologous micro-fragmented adipose tissue injection in diabetic patients' minor amputations. Thus, results from a 6-months randomized controlled single-center clinical trial named MiFrAADiF were analyzed aiming primarily to assess the impact on HRQoL and LOS.

Materials and methods

The MiFrAADiF was a randomized controlled single-center clinical trial. It was designed with two arms (parallel assignment) and no masking. The "treatment group" was intended as those patients assigned to local injection of autologous micro-fragmented adipose tissue and the "control group" as those assigned to the standard clinical practice following a minor amputation for DFU intended as digital or forefoot. All the phases of the trial including enrollment, treatment, follow-up visits, and data collection have been performed at the Department of Vascular Surgery of the Ospedale Civile di Baggiovara, University of Modena and Reggio Emilia, Modena, Italy. All the DFU patients who referred to our Diabetic Foot Service (hospitalized and outpatient) between April 7, 2015, and September 30, 2017, were screened for inclusion. The follow-up terminated on March 31, 2018. All the patients involved in the present trial had an angiographic assessment prior to the enrollment, and if required a revascularization by means of percutaneous transluminal angioplasty (PTA).

The flow diagram, the inclusion and exclusion criteria were previously published.[16] The specific diagram for the present outcomes is depicted in Figure 1. Adult patients (age >18 years) of both sexes presenting with diabetes mellitus (types 1 and 2) and irreversible DFU (digital and/or forefoot with ulcer and/or gangrene) were screened for study inclusion. The DFU was considered irreversible once complete wound healing was not achievable with conservative therapy and amputation was required. An

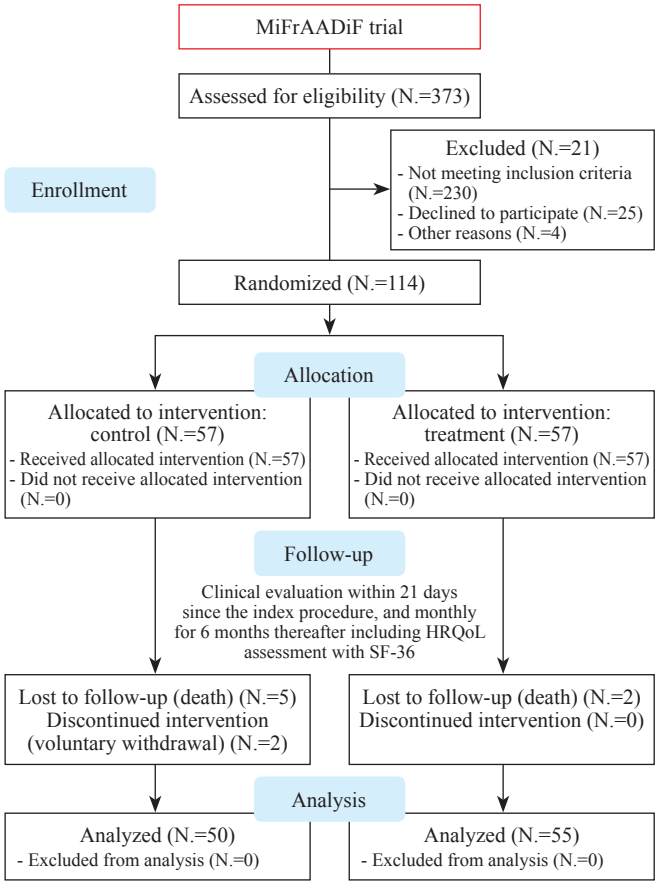

Figure 1.—Flow diagram of the MiFrAADiF Trial.

X-ray scan negative for osteolytic lesions was required. Other inclusion criteria, which ruled out peripheral artery disease were: an Ankle Brachial Index ≥0.7, a Pressure Finger/Arm Index and Toe/Brachial Index ≥0.6, a triphasic or biphasic doppler arterial waveform at the ankle level of the affected leg, and a foot transcutaneous oxygen test ≥30 mmHg.[17] Those patients who did not meet the above mentioned criteria were excluded. Additional criteria for exclusion were: ongoing or previous oncological treatments within the last 5-years, ongoing neoplastic lesions, ongoing corticosteroid therapy, pregnancy and/or lactation. Patients unable to speak Italian were not considered. Patients unable to sign the informed consent before entering the study were excluded. Other complications of diabetes such as cardiovascular disease and/or chronic kidney disease were not contraindications for the enrollment.

Regarding therapeutic management of diabetes, it was not a discriminating factor, oral hypoglycemic agents and insulin were both considered. Patients with a non-healing amputation at the end of the follow-up, stump dehiscence or an infection requiring reoperation were excluded for a second treatment and thus from the trial.[1]

Patients presenting with bilateral DFU were enrolled for a single limb, the contralateral was treated following standard care.

The Local Ethics Committee approved the trial (protocol number 2621/CE) after having reviewed the informed consent sheets and the case report form by the Local Ethics Committee (http://www.chirurgiavascolare.unimore.it/site/home/attivita-scientifica/articolo55471.html). The trial was registered in ClinicalTrials.gov (NCT03276312). It was conducted in compliance with the revised provisions of the Helsinki Declaration, in adherence with Good Clinical Practice, and following the current guidelines.[2-4]

Once a patient with DFU was referred to our Diabetic Foot Service, a clinical assessment was performed by our medical staff. A complete vascular physical work-up, and clinical examination of the lesions were performed as described above. Thus, a selected physician part of the research team screened the patients for inclusion criteria. All the participants signed the written consent. After the enrollment the baseline data were collected in the case report form (paper and electronical), which were kept confidential during each phase of the trial.

A 1:1 randomization to local injection of autologous micro-fragmented adipose tissue (treatment group) or to standard clinical practice (control group) was performed after a lower limb minor amputation. The details of the randomization process were previously published.[16] Briefly, a paper block system was used. Sheets in blocks of ten with five treatment assignment and the following five control assignment were sealed in a blank envelope. The envelopes were mixed and numbered 1 to 10. This process was performed by research assistants, part of the Internal Board of our Hospital. The investigators ignored the process used to create the assignments. After the first clinical screening, the Investigators collected the informed consent. The randomization of patients proceeded individually and was performed by the research assistants. Masking of the Physicians/Investigators or the patients was not possible for two main reasons: the harvest of adipose tissue is an invasive maneuver, and it is not ethically acceptable to perform it on a patient and then dispose of it, and it is a surgical procedure that needs a skilled surgeon. However, the statistician was masked to allocation during the analysis.

The detailed description of the surgical interventions was described before.[16]

All the patients involved in the study were treated in accor-

dance with current guidelines for diabetic foot problems.[2-4] All the trial procedures (surgery, fat processing, medications), and the clinical assessment (enrollment, follow-up visits, end of the study) were performed by the authors.

In both arms the amputations were performed under local anesthesia and the wound closed by primary intention. The medication of the stump consisted in a paraffin gauze with a povidone-iodine solution (10% of iodine) carried out after cleaning with sodium hypochlorite and saline solution.

The micro-fragmented adipose tissue injection (treatment group) was performed in the same surgical session immediately after the amputation. All the process took place in the operating theatre. The lipoaspiration technique was used to harvest the fat from the abdomen. The aspirated tissue was immediately processed in the Lipogems® processing kit (Lipogems International Spa; Milan, Italy) following a step-by-step procedure previously described in literature.[18] The kit was a single-use commercially available cylindric device. The mechanical force exerted by five stainless steel marbles (first reduction) reduced the size of the adipose tissue clusters and eliminated oily substances and blood residues. The mechanical-related trauma on the cells was minimized because of the complete immersion of the harvested tissue in the physiological solution.[18] The adipose clusters were dispersed a second time by passing through a reduction filter (clusters of 300-600 μm in diameter). The processed micro-fragmented fat was decanted, and the excess of saline solution was eliminated. At the end, the micro-fragmented fluid fat tissue product was injected in a radial pattern into the amputation wound through a 21 to 25-gauge caliber needle. The injected amount depended on the extension of the amputation wound (range 10-30 mL).[16] A compressive medication was applied for 24-48 hours on the site of harvesting. Absolute rest unloading the limb until complete wound healing was indicated to all patients.

In order to assess the HRQoL, the patients were asked to grade their quality of life through the Medical Outcomes Study 36-item Short-Form Health Survey (SF-36; http://www.qualitymetric.com). The SF-36 is a generic patient-recorded outcome measure (PROM) commonly used to test HRQoL in chronic diseases. It has been demonstrated to be effective for the assessment of DFU outcomes.[19, 20] Moreover, the SF-36 proved to be sensitive for temporal changes of HRQoL.[19] Two specific scores were provided, and consequently analyzed in the present work: the physical component summary (PCS), and the mental component summary (MCS). The values range from 0, meaning the maximum disability, to 100.

The LOS was considered as the hospitalization time elapsed between hospital admission and discharge.

The primary outcome was HRQoL comparison between groups, meaning the PCS and MCS analyzed by groups. The secondary endpoint was the comparison of the mean LOS between the groups.

The Ethical Committee monitored the trial for significant adverse events. In case of death, intervention-related complication, and life-threatening issues which may have been linked to the interventions, a notification was expected within 24 hours. The Committee required ad interim evaluations to monitor the intermediate results. Further definitions for clinical outcomes, including healing or failure, were published before.[16]

Statistical analysis

Based on our previous publication,[16] the statistical analyses plan was calculated under the assumption that the treatment provides a benefit of a 50% reduction in the healing time. A drop out of 10% was considered and the level of significance was set $\alpha=0.05$ with a power of 1; $\beta=0.80$. We calculated a total of 57 patients for each arm. One-way analyses of variance have been used to estimate the primary outcomes (PCS and MCS) differences by group. To take into account the repeated-measures nature of the primary outcomes the variable identifying the single patients was introduced in a paired analysis of variance. A two-way interaction between time and treatment was performed. A Tukey-Kramer pairwise comparisons (TK-test) was performed for each group to analyze the improvement of PCS and MCS during follow-up.

The LOS has been analyzed by Kaplan-Meier and log-rank test taking into account the discharge as the binary outcome. For all the analyses, statistical significance was assessed at two-sided 5% level ($P<0.05$) with 95% confidence interval (95% CI) and its standard error (SE). Results were reported to one decimal place. Analyses were based on the intention-to-treat principle using Stata 15.1 (Stata Corp; College Station, TX, USA).

Results

Between April 7, 2015, and September 30, 2017, we randomly assigned to microfragmented autologous adipose injection *vs.* standard care 114 patients with DFU. Each arm was accountable for 57 patients. A total of 373 patients were assessed for eligibility. The final 6-month follow-up was completed on March 31, 2018 (Figure 1). The cohort baseline information such as age, gender, smoke habits and comorbidities were published before.[16] All the patients re-

ceived endovascular revascularization before amputation. In the treatment group a below the knee PTA or femoral/popliteal plus below the knee PTA were performed in 77% (N.=44) and 23% (N.=13) of patients respectively. Patients being part of the control group received below the knee PTA or femoral/popliteal plus below the knee PTA in 74% (N.=42) and 26% (N.=15) of cases respectively.

Patients receiving an amputation for the first time were 49 (86%) and 53 (93%) in the treatment and control group respectively. The remaining were surgically treated at the site of a previous amputation, 8 (14%) being part of the treatment group *vs.* 4 (7%) being part of the control group. The amputation level was digital in 49 (86%) and transmetatarsal in 8 (14%), for both groups.[16]

Healing rate amongst patients completing the follow-up was 80% (N.=44/55) in treatment group and 46% (N.=23/50) in the control group. Patients with a non-healing wound in the treatment group were treated by revision of the digital stump (N.=9, 82%) or transmetatarsal amputation (N.=2, 18%). Patients with a non-healing wound in the control group were treated by revision of a digital stump (N.=19, 70%), revision of the forefoot stump (N.=1, 4%), transmetatarsal amputation (N.=5, 19%), or below the knee amputation (N.=2, 7%).

The primary outcome was the between-groups comparison of the HRQoL, especially the PCS and MCS. The mean values of the two scores at each follow-up visit are displayed in Table I.

Table I.—*Health-related quality of life outcomes.*

	Control group N.=57	Treatment group N.=57
PCS		
FUP visit 1	28.3 (26.9-29.7)	29.4 (27.9-30.8)
FUP visit 2	29.9 (27.9-31.8)	30.8 (29.0-32.6)
FUP visit 3	34.2 (31.4-36.9)	35.6 (33.3-37.9)
FUP visit 4	38.8 (35.5-42.1)	41.1 (38.5-43.8)
FUP visit 5	41.6 (38.2-44.9)	43.5 (40.9-46.2)
FUP visit 6	43.6 (40.1-47.0)	46.7 (44.1-49.3)
FUP visit 7	45.4 (41.9-48.8)	47.6 (44.9-50.2)
MCS		
FUP visit 1	33.9 (32.0-35.8)	35.2 (33.3-37.1)
FUP visit 2	37.2 (34.7-39.6)	39.1 (36.9-41.4)
FUP visit 3	41.5 (38.6-44.4)	43.5 (41.1-45.9)
FUP visit 4	44.6 (41.7-47.5)	46.1 (43.8-48.5)
FUP visit 5	45.3 (42.1-48.5)	47.5 (44.9-50.0)
FUP visit 6	47.5 (44.5-50.5)	48.3 (46.0-50.7)
FUP visit 7	48.5 (45.5-51.4)	49.5 (47.2-51.7)

The results are presented as means and 95% confidence intervals. Follow up (FUP).
FUP: Follow-up; PCS: health-related quality of life outcomes measured as physical component summary; MCS: health-related quality of life outcomes measured as mental component summary; SF-36: Medical Outcomes Study 36-item Short-Form Health Survey.

Treatment of the amputation stump with autologous micro-fragmented adipose tissue demonstrated a significant positive influence on PCS (P=0.001). When the time variable was included in a two-way interaction with treatment both resulted to influence the PCS (P=0.0000 and P=0.009, for time and treatment respectively). With respect to the baseline, the treatment group demonstrated a significant improvement at the third month of follow-up (PCS1 *vs.* PCS3, means 29.4 *vs.* 35.6; difference 6.3; TK-test 4.4; studentized range critical value: 4.2). The control group demonstrated a significant improvement at the fourth month of follow-up (PCS1 *vs.* PCS4, means 28.3 *vs.* 38.8; difference 10.5; TK-test 6.4; studentized range critical value: 4.2).

The MCS improvements resulted in a non-significant association when tested with paired analyses of variance (P=0.21). Time showed a significant influence on the MCS (P=0.0000). For the treatment group, a significant MCS improvement was registered at the second month (MCS1 *vs.* MCS2, means 35.2 *vs.* 39.1; difference 3.9; TK-test 4.3; studentized range critical value: 4.2). For the control group, the difference became significant at the third month (MCS1 *vs.* MCS3; means 33.9 *vs.* 41.5; difference 7.6; TK-test 7.4; studentized range critical value: 4.2).

The mean LOS was 16.2 days (95% CI: 13.8-18.7) and 24.4 days (95% CI: 17.4-31.3) for the treatment and the control group respectively. The LOS was assessed by means of Kaplan-Meier analyses and the log-rank test resulted significant (P=0.025) (Figure 2). The probability to be hospitalized for the treatment group at 10, 20, 30 and 40 days was 71.9% (SE=6.0; 95% CI: 58.3-81.8), 26.3%

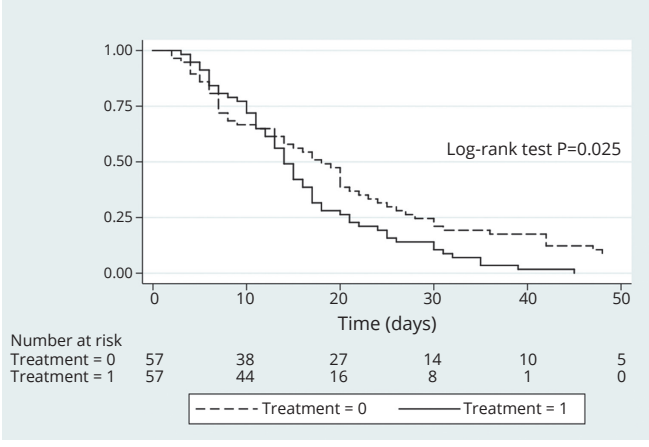

Figure 2.—Kaplan-Meier analysis of the hospitalization length of stay and its log-rank test.
Treatment =0 stands for the control group; treatment =1 stands for the treatment group.

(SE=6.0; 95% CI: 15.8-38.1), 10.5% (SE=4.1; 95% CI: 4.3-20.0) and 1.8% (SE=1.7; 95% CI: 0.1-8.2) respectively. For the control group, the probability at 10, 20, 30 and 40 days was 64.9% (SE=6.3; 95% CI: 51.1-75.7), 38.6% (SE=6.5; 95% CI: 26.1-50.9), 21.1% (SE=5.4; 95% CI: 11.6-32.4) and 12.3% (SE=4.3; 95% CI: 5.4-22.2) respectively.

Discussion

HRQoL and LOS were improved in diabetic patients receiving local micro-fragmented autologous adipose tissue injection after minor amputation compared to standard care.

However, several points required discussion. Physical health perception resulted in a significant positive association with treatment (P=0.001). With regard to mental health, the association with treatment was not significant. The time elapsed after treatment demonstrated a remarkable statistical significance with PCS and MCS. For this reason, we compared the intra-group scores to baseline at each follow-up visit. The treatment group reported a significant improvement at the third and the second follow-up month visit for the PCS and MCS respectively. The control group reported a significant improvement at the fourth and the third follow-up month visit for the PCS and MCS respectively. In other words, patients being part of the treatment group reported better physical results. Thus, patients in the treatment group demonstrated greater improvements in PCS and MCS on follow-up than patients in the control group (Table I). Apparently, the adipose tissue harvesting process was not negatively perceived by the patients probably due to the low invasiveness of the liposuction. Besides, the healing rate was much higher in the treatment group allowing early mobilization and the capability to complete daily living activities.[16] As depicted, these were the factors influencing the physical quality of life in the present trial.

The MiFrAADiF Trial demonstrated a significant LOS reduction in the treatment group *vs.* standard care (16.2 *vs.* 24.4 days; P=0.0000). These results could be considered comparable to those presented in a large longitudinal study which encompassed more than 27,000 diabetic patients reporting a mean 21.6 days LOS after amputation.[21]

The MiFrAADiF Trial was the first RCCT to demonstrate improved healing rate in such patients, and the results were published before by our group. The present publication of the trial validated the efficacy of this new technique on the quality of life and the LOS. To the best of the authors' knowledge this is the first trial to study such outcomes in diabetic patients with foot ulcers.

In order to discuss the HRQoL in diabetic patients several issues need to be pointed out. The SF-36 was a PROM commonly employed in literature.[19, 22] Among the plenty of PROMs available in literature there are no gold standard tests to universally measure patients' health outcome.[19, 22] The Italian version of the SF-36 has not been evaluated specifically for diabetes mellitus, nevertheless it has been widely used during the last two decades to evaluate patients' related outcomes in different settings.[23] This is an important aspect, considering that culture and ethnicity of the patients have been demonstrated to impact the self-reported HRQoL questionnaires in both type-1 and type-2 diabetes.[24, 25] Moreover, those patients presenting with DFU resulted in lower baseline HRQoL scores.[9, 20] Ribu *et al.*[26] underlined that even a slight improvement in physical functioning should be considered relevant taking into account the important limitations experienced by DFU patients. Our PCS results (means 47.6 *vs.* 45.4; treatment *vs.* control respectively) were superior compared to the mean 35.5 previously reported at 6-month in healed DFU.[27] This appeared to support the choice to perform a minor amputation instead of conservative treatment taking into account that patients receiving a minor amputation had already demonstrated better physical outcomes compared to active DFU patients.[10, 28] As demonstrated before, a low PCS is a significant predictor of patients mortality so every effort should be made to improve their HRQoL and PCS.[29] We believe that all the above presented aspects would emphasize the HRQoL results coming from the present trial.

Mental health is also an important issue when HRQoL in diabetic patients with DFU is to be determined. The MCS results coming from the MiFrAADiF trial were slightly comparable with those found by Ribu *et al.*[27] (48.5 and 49.5; control and treatment group respectively *vs.* 46.9). As above, the mentioned results were found at 6-month in healed DFU. In our trial, the MCS improvements were significant when tested with one-way analyses of variance. However, the significance was lost when a paired test was run. The root cause remains unknown and many speculations on the related cofounders could be made. A possible explanation could be related to disease awareness and introspection ability of elderly and diseased patients. It is known from the literature that diabetic patients are not always aware of the whole specter and the magnitude of possible complications of the disease.[27] Diabetic patients also tend to fear amputation as the worst diabetic complication instead of death after they have experienced foot ulcers.[27] To paraphrase, the major fear of diabetic patients was death before the onset of ulcers, while later on was

the risk of amputation. However, this change of perceptions does not imply a change of the real risks. We have also observed that patients had greater difficulties filling in the MCS-related items compared to those related to the PCS. Advanced age, diabetes, comorbidities, along with the possible underlying cognitive impairment, could have influenced the introspection ability resulting in biased results. In our opinion it would be interesting to compare an external psychological assessment of the mental status with the self-reported awareness of the disease in order to characterize possible differences.

Limitations of the study

The present trial has potential limitations. The main limitation is that a double-blind study could not be performed because it was ethically unacceptable to harvest adipose tissue from patients in the control group without further use. The small sample size represents an issue, although it was statistically determined. We employed the SF-36, whose specific limitations were described above. Amongst the plethora of PROMs available, studies reporting minor amputation SF-36 outcomes have been lacking and a direct comparison was not feasible. We compared our results with those reported in healed DFU. However, it should be considered that we treated patients that had already undergone unsuccessful conservative treatments. A number of specific limitations and future perspectives of the autologous adipose tissue and its mesenchymal stem cells were emphasized in our previous publication.[16] A larger multicenter trial would be required to confirm the present results.[30] The impact of the LOS reduction on the overall costs of care represents an additional point of interest for health care providers. Future investigations are required on this specific topic. Different aspects concurred to increase the LOS such as the revascularization procedure performed during the same hospitalization, the availability of an operative room for the amputation, and the cares provided together with Internal Medicine Colleagues to deal with the broad spectrum of comorbidities. Nevertheless, these possible confounders were equally distributed between groups and did not bias the difference found.

The results of the present trial are relevant, and the injection of micro-fragmented autologous adipose tissue should be considered an effective technique to improve healing and physical functioning of these highly diseased patients. This treatment could have several benefits avoiding some potential limb-threatening complications related to conservative treatments such as worsening of the wound, infections, prolonged non-ambulatory time, and prolonged LOS.[31]

In everyday practice the clinical dilemma regarding the need and timing of amputation in patients with chronic DFU is always present.[10, 32] A tool that would fasten an amputation wound healing and improve the physical and mental wellbeing of the patients could help in this hard decision making process. A multidisciplinary team with the knowledge and skills of using different treatment options for patients with DFU could offer better outcomes to the patients thus reducing also the medical burden and costs.[27, 32]

Conclusions

The MiFrAADiF Trial demonstrated the improvement of health-related quality of life and shortening of hospital length of stay after micro-fragmented autologous adipose tissue injection in the minor amputation bed performed in case of diabetic foot ulcers. This new technique and its operators will usher in a new treatment paradigm for minor amputation in diabetic patients.

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