Updates Surg (2017) 69:523–529
DOI 10.1007/s13304-017-0461-9

ORIGINAL ARTICLE

To evaluate the efficacy of an acellular Flowable matrix in comparison with a wet dressing for the treatment of patients with diabetic foot ulcers: a randomized clinical trial

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Received: 14 February 2017 / Accepted: 1 May 2017 / Published online: 11 May 2017

Abstract The authors aimed to evaluate the efficacy of an advanced wound matrix (Integra Flowable Wound Matrix, Integra LifeScience Corp, Plainsboro, NJ, USA) for treating wounds with irregular geometries versus a wet dressing in patients with diabetic foot ulcers. Sixty patients with diabetic foot ulcers (Grades 3 Wagner) were included in this randomized clinical trial. The study was conducted in the General Surgery Unit and Geriatric of the Second University of Naples, Italy, in the last 12 months. Forty-six cases of diabetic foot ulcers were equally and randomly divided into control and test groups. The first group treated with Integra Flowable Wound Matrix, while the control group with a wet dressing. Both groups were evaluated once a week for 6 weeks to value the degree of epithelialization and granulation tissue of the wound. The complete healing rate in the whole study population was 69.56% (Integra Flowable Wound Matrix group, 86.95%, control group, 52.17%; $p = 0.001$). Amputation and rehospitalization rates were higher in the control group compared to the first group, therefore, the difference was statistically significant ($p = 0.0019$; $p = 0.028$, respectively). The Integra Flowable Wound Matrix, was significantly superior, compared to the wet dressing, by promoting the complete healing of diabetic foot ulcers. Ease of use, absence of adverse effects, and a facilitated wound healing process are among the properties of the matrix. These characteristics make it appropriate in the management of diabetic foot ulcers. Additional research will shed more light on the promising advantages of this material in healing diabetic foot ulcers.

Keywords Diabetic foot ulcers · Tunneling lesions · Biomaterial · Flowable matrix

Abbreviations

DFUs	Diabetic foot ulcers
TNF	Tumor necrosis factor
IL-1b	Interleukin 1 beta
VEGF	Vascular endothelial growth factor
MMPs	Matrix metalloproteinases
PPAR	Peroxisome proliferator-activated receptors
IFWM	Integra TM Flowable Wound Matrix
ABI	Ankle-brachial index
CI	Confidence interval
MDNS	Michigan Diabetic Neuropathy Screening Score
MIC	Minimal inhibitory concentration
PP	Per-Protocol

Introduction

In diabetic patients, frequently, the wound is found to "tunnel" into deep soft tissue with involvement of bones and tendons. A diabetic foot ulcer is a pivotal event in the life of a person with diabetes and is one of the complications of diabetes that can cause life threatening. Diabetic foot ulcers have a major economic impact as well; data have shown diabetic foot ulcers (DFUs) are a major cause of hospitalization for patients with diabetes [1]. Without early and optimal intervention, the wound can rapidly deteriorate, leading to amputation of the affected limb.

Optimum healing of a cutaneous wound requires a well-orchestrated integration of the complex biological and

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molecular events of cell migration and proliferation, and of extracellular matrix deposition and remodeling. Cellular responses to inflammatory mediators, growth factors, and cytokines, and to mechanical forces, must be appropriate and precise. In the diabetic wound, intrinsic pathobiological abnormalities and extrinsic factors contribute to an even more complex wound microenvironment. Clinical and experimental evidence suggests that diabetic ulcers do not follow an orderly and reliable progression of wound healing. Parts of the chronic wound may be stuck in different phases, having lost the ideal synchrony of events that leads to rapid healing. Some of the resident cells in diabetic ulcers become phenotypically altered: macrophages in diabetes showed a decrease in release of the cytokines including TNF-α, IL-1b and VEGF [2].

Over 100 known physiologic factors contribute to wound healing deficiencies in individuals with diabetes. These include decreased or impaired growth factor production, angiogenic response, macrophage function, collagen accumulation, epidermal barrier function, quantity of granulation tissue, keratinocyte and fibroblast migration and proliferation, number of epidermal nerves, bone healing, and balance between the accumulation of extracellular matrix components and their remodeling by MMPs [3].

Technologies for various molecular analyses, novel discoveries of disease molecular pathogenesis from studies of patient biopsies and animal models, and major advances in tissue engineering, could potentially be applied to people with diabetic wounds in the near future. New treatments for diabetic foot ulcers continue to be introduced, but few are subjected to controlled trials [4–8].

Peroxisome Proliferator-Activated Receptors (PPAR) play a key role in metabolic diseases, which include several cardiovascular diseases, insulin resistance, type 2 diabetes, metabolic syndrome, impaired immunity and the increasing risk of cancer; PPAR agonists could represent interesting types of molecules that can treat not only metabolic diseases, but also inflammation and cancer [9]. Furthermore, PPARδ plays pivotal roles in wound healing by promoting fibroblast-to-myofibroblast differentiation via transforming growth factor; GW501516-activated PPARδ increases the migration and contractile properties of human dermal fibroblasts and upregulates the expression of myofibroblast markers such as collagen I and fibronectin [10–12].

Other biopolymers have been used for some years in the treatment of chronic skin lesions as a scaffold for regenerating new tissue in situ [13]. Skin substitutes represent the pinnacle of bioengineering techniques and are poised to offer an exciting new treatment strategy in complex wound management [14]. The aim of these products to provide a scaffold for cellular and vascular in-growth and promote wound healing. The dermal substitutes must behave like the extracellular matrix to form a template for host infiltration and a physical support to guide the differentiation and proliferation of cells involved in cutaneous wound healing [15, 16]. The dermal substitutes induce the influx of endogenous cells including fibroblasts, keratinocytes, endothelial cells, macrophages, and neutrophils into the wound bed. These cells then secrete a variety of cytokines and growth factors that stimulate angiogenesis, extracellular matrix deposition and, re-epithelialization via the process of dynamic reciprocity. Within the dermal template, host fibroblasts migrate, proliferate and then secrete a native collagen. Endothelial cells shortly follow the fibroblasts to form a vascular network within the neodermis [17].

Skin substitutes function as sterile tissue grafts that are applied directly to a wound bed and integrate with the surrounding native tissues to actively stimulate cell migration, angiogenesis, and epithelialization, resulting in accelerated wound healing [18]. The clinical use has highlighted problems with applying dermal substitutes to irregularly shaped wound beds and in particular tunneling wounds. The injectable matrices in the form of flowable gels or pastes have emerged and are an exciting concept for cutaneous repair and regeneration.

Integra TM Flowable Wound Matrix (IFWM) has a fluid composition and a method of application which allows complete filling of deep cavities and/or tunneling wounds. The biomaterials, in fact, must conform to the lesion and come in contact with the bed and the walls of the cavity; otherwise, colonization and vascularization would be inhibited.

The matrix Integra TM Flowable Wound Matrix, consisting of cross-linked Type I collagen, glycosaminoglycans and glycoproteins, provides resorbable scaffold. The granules of collagen of type I, of bovine origin, represent 90% of the structure have a size between 200 and 2000 microns. These constitute a three-dimensional scaffold with a variable microporosity (10–500 microns) to ease the migration of cells into the matrix, allowing an effective remodeling of the same [8]. The glycosaminoglycans in the matrix, are represented by the chondroitin-6-sulfate (10% of the structure), while the glycoproteins are fibronectin, laminin, and elastin, chondronectin.

In the current prospective study, the authors aimed to assess the possible efficacy and tolerability and the safety of Integra TM Flowable Wound Matrix in diabetic patients with lesions involving deep structures in comparison with a wet gauze dressing.

Materials and methods

Study design and participants

The present study was a randomized placebo-controlled clinical trial. The trial was carried out in compliance with the principles laid down in the Declaration of Helsinki, in

accordance with the international conference on harmonization good clinical practice guideline, and in accordance with applicable regulatory requirements. All patients signed the written informed consent form after receiving adequate information about the study. The Institutional Review Board of the Second University of Naples approved the study protocol.

This randomized clinical trial was conducted in the "Centre for the study and treatment of skin lesions and diabetic foot of General Surgery" at the General Surgery Unit and Geriatric of the Second University of Naples, Italy, in the last 12 months. Sixty patients with diabetes who had a Grades 3 Wagner ulcer were assessed for eligibility in this randomized clinical trial.

Inclusion criteria were male and female patients with diabetes >18 years of age, who had DFUs with Grade 3 Wagner classification; who had an ankle-brachial index (ABI) of ≥0.5;

Patients excluded from the study were those with coagulation disorders and autoimmune diseases or cardiopulmonary diseases, with poorly controlled diabetes (HbA1c ≥ 10%), or with a Michigan Diabetic Neuropathy Screening Score (MDNS) ≤3; pregnant women; smokers; and recreational drug users.

Neuropathy was assessed by means of the MDNS. Peripheral neuropathy was present when the MDNS score ≥three-eighths [19]. The vascular assessment was performed based on clinical examination and ABI measurement. Patients with an ABI < 0.8 were considered to have peripheral vascular disease [20].

All patients underwent clinical examination and a full medical history was obtained. The results of paraclinical assessment as well as the characteristics of the wounds were recorded.

Treatments

Participants were then randomized into two groups using a simple randomization method: one group was treated with Integra Flowable Wound Matrix and the other group was treated with the wet dressing as control.

All patients were subjected to biopsy of the lesion to determine bacterial culture with antibiogram and Minimal Inhibitory Concentration (MIC). Targeted Antibiotic treatment started 7–10 days before surgery, depending on the type of bacteria, and continued until the wound healing.

During each examination of patients in both groups, wounds were first washed with a normal sterile saline solution, and then necrotic tissues were removed surgically by hydro-scalpel (Versajet, Smith & Nephew) until normal, healthy tissues appeared.

In the first group, the Integra Flowable Wound Matrix was applied directly to the wound bed.

After mixing the dry granular collagen with saline solution, the matrix Integra TM Flowable Wound Matrix was applied to the lesion, using the flexible injector, until completely full. The edge of the wound was sutured with silk 2.0/3.0.

Wounds in the control group were covered with sterile saline-moistened gauze before the dressing. The surgical breach healed by secondary intention.

The patients underwent different anesthesia (spinal anesthesia or ankle block).

An inelastic multilayer multicomponent bandage was used as compression therapy in both groups. It was recommended to patients not to walk and upload for 2 weeks; subsequently, a limited ambulation was allowed using a removable offloading device.

Patients were examined weekly up to a maximum of 6 weeks (42 ± 2 days) if complete wound closure had not occurred. In cases of complete wound healing before 6 weeks, follow-up was ceased. During the first week, follow-up visits were performed every 3 days.

At each follow-up, general and local clinical examination was performed: the presence or absence of any clinical sign of inflammation (edema, erythema, increased local temperature, the presence of abscess) was recorded. Every 10 days, the patients were subjected to laboratory investigation, with evaluation of inflammation index VES, PCR and fibrinogen. A radiographic exam (Rx and MRI) of the foot was performed to evaluate the involvement of deep structures before and after surgery (30 and 60 days after treatment). In the next step, the wounds were photographed with a digital camera.

The state of healing was assessed by clinical examination, and final healing was defined as complete re-epithelialization of the wound in the absence of discharge.

Any potential diabetes-related complication (e.g., infection, necrosis, or an allergic reaction) was recorded.

End-points

The primary objective of the study was to determine the percentage of patients with Wound closure (was defined as 100% re-epithelialisation) in their wounds or wound closure within the 6-week study period. Secondary endpoints included the time to healing into two groups. Reported safety endpoints included a number of major amputation and number of hospitalization.

Statistical analyses

Data were analyzed using SPSS version 16 for Windows (SPSS Inc, Chicago, IL). The continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequencies (%). The mean

difference of continuous variables between groups was assessed by Student t test. The comparisons of qualitative variables between groups were performed by χ^2 test. The time healing distribution was estimated by Kaplan–Meier, compared by log-rank test. Median time (i.e., ulcer closure for 50% of participants) was calculated using a 90% CI. A p value of <0.05 was considered statistically significant.

Results

Forty-six patients were then matched and assigned to receive Integra Flowable Wound Matrix (Integra LifeScience Corp, Plainsboro, NJ) or wet dressing. Participants were then randomized into two groups using a simple randomization method: one group ($n = 23$) was treated with Integra Flowable Wound Matrix and the other group ($n = 23$) was treated with the wet dressing as control.

The flow diagram shows the study design (Fig. 1).

Only randomized patients, who had complied with the study protocol, were analyzed (analyses of the PP population); no patient was lost to follow-up.

Baseline demographics and clinical characteristics at randomization were comparable between treatment groups (Table 1).

A total of 46 patients with diabetes (28 males and 18 females) with a mean age of 63.06 ± 8.32 years were recruited in this study. There were no statistically significant differences in respect to demographic or baseline clinical presentations between the two groups (Table 1).

The mean duration of ulcer development was 38.56 ± 12.61 weeks in the Integra Flowable Wound Matrix group and 39.5 ± 9.90 weeks in the wet dressing group.

The most frequently selected bacteria were Staphylococcus aureus in 56.32%, Pseudomonas aeruginosa in 47.83%, Enterobacter cloacae in 15.42%, Staphylococcus epidermidis in 17.50%, Proteus mirabilis in the 7.68% and the Streptococcus β- haemolytic in 6.87%.

The patients underwent different anesthesia: spinal anesthesia in 7 patients (15.21%) and ankle block in 39 patients (84.78%).

Minor amputations were performed: nine (39.13%) patients in the first group and eight (34.78%) patients in the control group.

After 6 weeks, the overall complete healing rate among all patients was 69.56%. Complete wound healing after 6 weeks (primary endpoint), occurred in 20 patients (86.95%) of the Integra Flowable Wound Matrix group and in 12 patients (52.17%) of the control group. A significant difference in complete healing was observed between the two groups after 6 weeks [relative risk (RR) 1.67; 95% CI 1.09–2.54, $p = 0.01$] (Table 2).

After 6 weeks, 3 of the 23 patients (13.04%) in the first group showed no healing, without any clinical sign of inflammation (edema, erythema, increased local temperature, the presence of abscess) and/or laboratory signs of inflammation (VES, PCR and fibrinogen).

Time to healing (secondary endpoint) was between: 29.73 ± 9.27 days in the first group and 42.78 ± 8.22 days in the control group. The time healing distribution was estimated by Kaplan–Meier, compared by log-rank test ($p < 0.000$) (Fig. 2).

Major amputation and rehospitalization rates (safety endpoints) were higher in the control group compared to Integra Flowable Wound Matrix group, these differences reach a significant level (relative risk RR 0.16; 95% CI 0.02–1.17, $p = 0.028$; relative risk RR 0.10; 95% CI 0.01–0.72, $p = 0.019$, respectively) (Table 2).

Discussion

In the present study, the authors evaluated the efficacy and safety of advanced wound matrix for treating wounds in patients with diabetic foot ulcers.

A significantly higher percentage of patients (86.95%) in the Integra TM Flowable Wound Matrix group achieved complete wound healing in 6 weeks, compared to the patients in the wet dressing group (52.17%). Deep structures were quickly covered with regenerated tissue.

In 13.04% of patients there is no wound healing, after 6 weeks. These patients with graft failure had no clinical and/or laboratory signs to report to the inflammatory process inside. In our opinion, this dermal graft failure could be due to an inappropriate surgical timing in the first two patients; in the third patient, engraftment failure could be due to a less aggressive debridement.

Healing time was significantly shorter in the first group, where the surgical breach was closed by primary intention compared to the control group, where the surgical breach healed by secondary intention. The biomaterial allows us to close the wound by primary intention, reducing the healing time.

In the present study, the rate of complications such as major amputation and rehospitalization was lower in the Integra TM Flowable Wound Matrix group compared with the wet dressing: this difference was statistically significant.

The main role of surgery, so far, has been represented by the removal of infected or necrotic, tissue, until healthy tissue induced the formation of granulation tissue and healing by second intention.

The use of biomaterials in expert hands and in selected patients allows a quick natural healing of the lesion and allows the decrease of major amputations with more distal amputations in the lower limbs. Tissue engineering recently

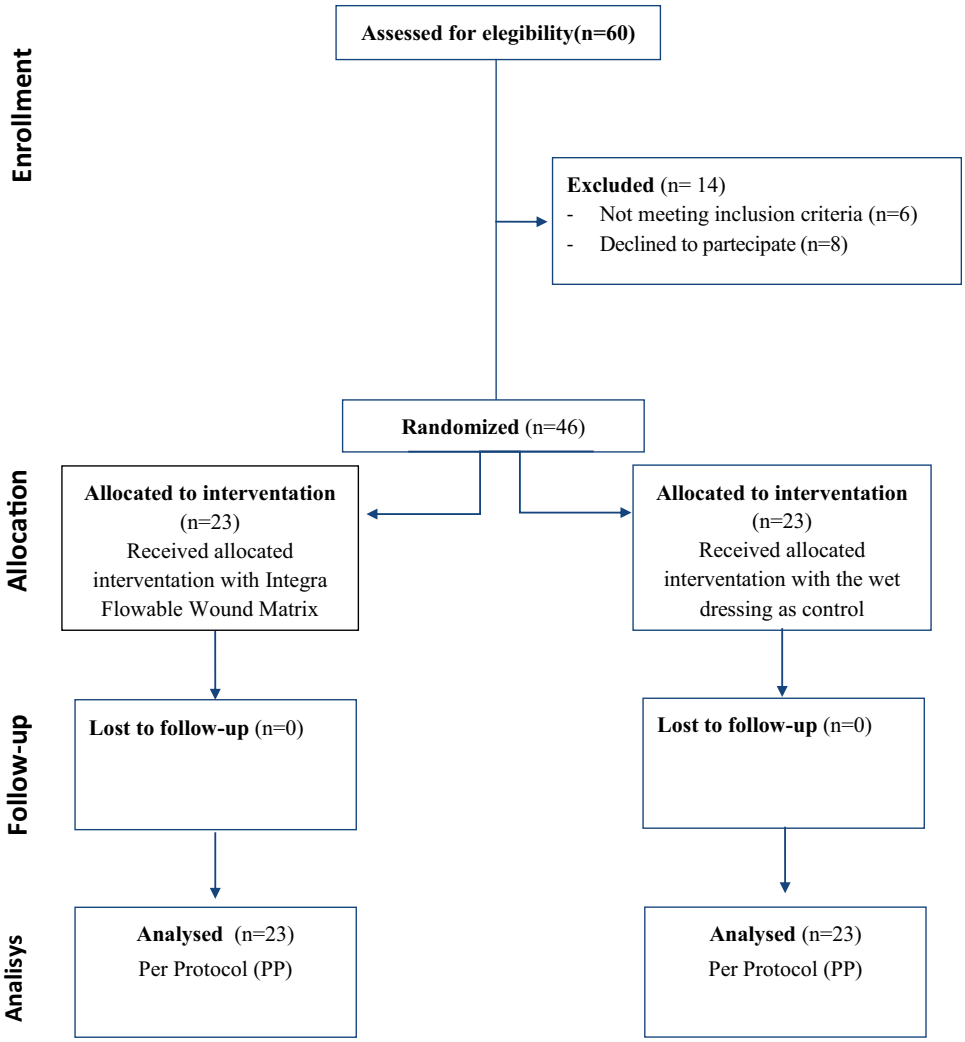

Fig. 1 Consort flow diagram of participants

Table 1 Baseline demographic data of patients

Baseline characteristics	Integra Flowable Wound Matrix ($n = 23$)	Control group ($n = 23$)	p value*
Age (years)	64.04 ± 8.94	62.08 ± 7.71	0.210
Gender (male/female)	8 females (34.78%)	10 females (43.47%)	0.54
	15 males (65.22%)	13 males (56.53%)	
Body mass index (kg/m^2)	28.47 ± 2.48	28.91 ± 2.67	0.14
HbA1c (%)	7.9 ± 0.8	7.8 ± 0.8	0.5
Ankle-brachial index	Right: 0.92 ± 0.1	Right: 0.94 ± 0.1	0.25
	Left: 0.92 ± 0.1	Left: 0.93 ± 0.1	0.4
Ulcer type	Neuropathic: 19 (82.60%)	Neuropathic: 17 (73.91%)	0.47
	Neuroischemic: 4 (17.39%)	Neuroischemic: 6 (26.09%)	
Ulcer location	Abscesses foot: 18 (78.26%)	Abscesses foot: 16 (69.56%)	0.75
	Heel: 1 (4.35%)	Heel: 2 (8.69%)	
	Metatarsal head: 4 (17.39%)	Metatarsal head: 5 (21.74%)	
Mean duration of ulcer (weeks)	38.56 ± 12.61	39.5 ± 9.90	0.75
Osteomyelitis	9/23 (39.13%)	8/23 (34.78%)	0.76

* $p < 0.05$ were considered significant

Table 2 Comparison of outcomes between the two groups

	IFWM group	Control group	Rate ratio	95% CI	*p* value*
Primary endpoint					
Complete healing after 6 weeks	20 (86.95%)	12 (52.17%)	1.67	1.09–2.54	0.010
Safety endpoint					
Rehospitalization	2 (8.69%)	10 (43.47%)	0.10	0.01–0.72	0.001
Major amputation	1 (4.34%)	7 (30.43%)	0.16	0.02–1.17	0.028

CI confidence interval

* $p < 0.05$ were considered significant

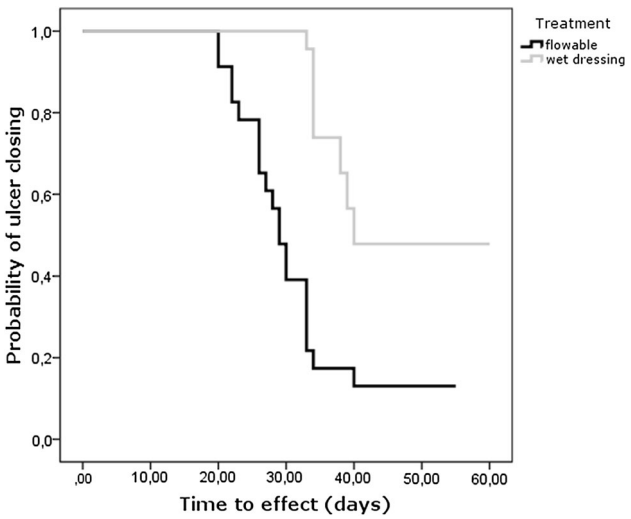

Fig. 2 Kaplan–Meier plot of time to 100% ulcer closure with IFWM compared to control

provided cellular and acellular biomaterials in sheet form suitable for the regeneration and repair of flat skin lesions [21–24], reducing major amputations, the risk of surgery and healing time. The use of these materials in sheet form; however, is limited by the impossibility of application in the cavity and/or tunneling lesions [25–28]. These limits seem to overcome by new Flowable biomaterials capable of filling the cavities after debridement and expanding avoiding dead spaces [29]. The Integra TM Flowable Wound Matrix is a "biological graft" that is particularly useful for the treatment of tunneling skin lesions, which cannot be treated applying a dermal substitute in sheet form [27]. Integra TM Flowable Wound Matrix is a biomaterial that, once hydrated with saline, becomes sufficiently fluid to be applied into the deep lesions and/or with irregular geometry. The expanding of collagen particles, after hydration, allows a more intimate contact of the matrix with the wound bed, and a more complete coverage of deep lesions, thus providing a support for the cellular invasion and capillary growth. Our experience shows how the fluid matrix can be applied easily (like a gel), without the need of donor sites, or any additional risks for the patient. After the application it expands, filling the volume of the lesion completely, absorbing tissue fluid and stopping the inflammation; this biomaterial is recognized as self by the immune system; it does not attract platelets and white blood cells and it stimulates the host response to the regeneration instead [26].

Conclusion

The results of the present study demonstrated the biomaterial tested can be considered an effective adjunctive treatment in the promotion of wound healing in the patients with DFUs.

The biomaterial used in this study allows the treatment of tunneling/or cavity lesions with an irregular geometry of the diabetic foot, which cannot be effectively treated using other biomaterials in the sheet form.

Furthermore, easy application, the absence of adverse effects and a minimally invasive approach by primary intention closure of the lesion, make it appropriate in the management of DFUs.

However, further research is warranted to shed more light on the advantages of this biomaterial.

Acknowledgements The authors would like to acknowledge the Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences for providing patients and necessary staff for this study.

Compliance with ethical standards

Conflict of interest The authors declare the absence of any potential conflict of financial interest (or none) for all authors.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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