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Original research

Assessment of bacterial infection in chronic wounds in the elderly: Biopsy versus VERSAJET

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ABSTRACT

Aim: The aim of this study was to evaluate the hydro-surgery VERSAJET system as a suitable alternative to the traditional invasive tissue sampling technique in detecting bacteria and their load in chronic wounds in the elderly. **Materials and methods**: To investigate and evaluate bacterial incidence and load in chronic wounds, we simultaneously performed on 19 affected patients a deep tissue biopsy and tissue collections by the VERSAJET hydro-surgical system. After local cleaning and anesthesia, a deep biopsy was performed with a punch of 3–4 mm in diameter. Subsequently, three tissue samples were collected by the VERSAJET system: one from the first washing in order to investigate the superficial contamination; one from the second washing to investigate deep tissue infection investigation and one from the third washing as a control procedure. After treatment, all tissue samples were cultured in vitro for diagnostic and micro-biological assessment. **Results**: Nineteen patients with chronic wounds of the lower limbs were enrolled from February 2010 to May 2013. Concordance between deep tissue biopsy cultures and tissue cultures collected by the VERSAJET system was examined. The deep tissue biopsy cultures showed complete concordance with the VERSAJET as follows: 2 patients (11%) for the first washing sample; 10 patients (53%) for the second washing sample; 4 patients (21%) for the third washing sample. However, with reference to only aerobic isolated strains, the concordance of the VERSAJET second washing samples cultures with a biopsy of the deep tissue cultures was very high (84%) and fairly high (63%) in the anaerobic isolated strains. The second VERSAJET washing sample cultures seem to have the highest concordance with the biopsy of the deep tissue cultures. **Conclusions**: Tissue biopsy remains the leading technique for detecting bacteria and their load in chronic wounds. However, this study shows that the hydro-surgery VERSAJET system is sufficiently effective in detecting bacteria and their load in chronic wounds and can be a potential alternative to a biopsy. In particular, the second washing sample culture showed the best correlation with the deep tissue biopsy culture. However, further studies are needed in order to modify techniques of tissue collection in the VERSAJET system before drawing any conclusions.

1. Introduction

Aerobic and anaerobic bacteria play an active and critical role in the chronic wound pathogenesis [1]. Frequently, lesion progression towards chronicity is related to an increase in affected tissue of bacteria load [2]. As the bacterial load increases so will the wound healing time. The debridement is essential for the wounds healing and several techniques are available to remove the necrotic tissue and reduce bacterial load to convert a chronic into an acute wound.

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The surgical debridement of the necrotic tissue is the gold standard and is needed for treating the wound; however, this practice, even if carried out quickly, can be painful and non selective because it can remove healthy tissue. A variety of mechanical methods for wound debridement are being evaluated and one of the most promising is the hydro-surgery system (VERSAJET) [3].

The VERSAJET is a debridement tool based on the Venturi effect which simultaneously produces demolition and removal of the necrotic tissue and drastically reduces bacterial load [5]. This technology uses a sterile water jet at variable power settings [4,5] and is better tolerated by patients. The deep tissue sampling by biopsy is an invasive technique, which requires technical skills and is unpleasant to patients [6]. Therefore, evaluation of the VERSAJET usefulness in performing specimens for both diagnostic and microbiological assessment appeared very interesting.

The aim of this study was to evaluate the VERSAJET usefulness in detecting bacteria and their load in chronic wounds in the elderly.

2. Materials and methods

2.1. Our population

Nineteen patients affected by chronic wounds on the lower limbs with evident infection supported by laboratory findings (presence of necrotic tissue, purulent exudates, neutrophilic leukocytosis, etc.) afferent to the Medical Angiology Department of the Second University of Naples were enrolled.

All patients signed the form for informed consent.

2.2. Tissue sampling

To investigate and evaluate the bacterial load, before any antibiotic therapy or ten days after antibiotic withdrawal, we simultaneously performed on patients, both a biopsy of the deep tissue and three tissue samples by hydro-surgical VERSAJET system.

The deep tissue biopsy was performed with a punch of 3–4 mm in diameter after local anesthesia and cleaning. The specimen was placed in a sterile container with screw closure, without fixation [7,8].

The VERSAJET sampling was performed after wound surface cleansing with power set at 3 as follows:

- A primary collection of 100 ml of liquid (first washing on the whole ulcer area with power set to 5) to investigate on superficial contamination.
- A second collection of 250 ml (second washing on the edges and at the center of the lesion with power set at 7 or more) to investigate on deep tissue infections.
- A third collection (third washing, as the second one, but set at the maximum tolerated power of 9 or 10) as control procedure control and standardized support.

All three samples were collected in sterile containers and sent, together with the biopsy tissue, to the Microbiological Department of the Second University of Naples.

The time lapse from specimens sampling and processing never exceeded 20 min.

2.3. Specimens processing

All steps were performed under laminar flow hood.

2.3.1. Deep tissue

Two grams of deep tissue were aseptically transferred in a 15 ml test tube and ground with a sterile tissue-milling machine (Griffiths test tube) and then homogenized adding 1 ml of sterile Brain Hearth Infusion broth. Homogenate was diluted with sterile Brain Heart Infusion broth to the final volume of 5 ml and again homogenized by the vortex for 10–15 s.

Table 1
Basal characteristics of patients.

Characteristics of patients	Venous ulcer ($n = 8$)	Arterial ulcer ($n = 6$)	Ulcer mixed ($n = 5$)	Total ($n = 19$)
Gender, no. (%)				
Male	3 (38%)	2 (33%)	1 (20%)	**6 (31.6%)**
Female	5 (62%)	4 (67%)	4 (80%)	**13 (68.4%)**
Age (years)				
Median	68	70	68	**68.5**
Range	*(65–71)*	*(67–73)*	*(67–69)*	***(65–73)***
Smokers, no. (%)				
Yes	5 (62%)	4 (67%)	5 (100%)	**14 (73.7%)**
No	3 (38%)	2 (33%)	–	**5 (26.3%)**
Comorbidities, no. (%)				
Diabetes mellitus type 2	1 (12.5%)	–	–	**1 (5.2%)**
Hypertension	2 (25%)	6 (100%)	2 (100%)	**10 (52.6%)**
Ischemic heart	2 (25%)		1 (20%)	**3 (15.8%)**
Peripheral vascular disease	1 (12.5%)	6 (100%)	2 (40%)	**9 (47.4%)**
Degenerative arthropathy	2 (25%)	2 (33%)	1 (20%)	**5 (26.3%)**
Metabolic disorders	8 (100%)	4 (67%)	3 (60%)	**15 (78.9%)**
Other	3 (38%)	2 (33%)	2 (33%)	**7 (36.8%)**

2.3.2. VERSAJET samples

Each washing liquid specimen was shaken by the vortex for 10–15 s and vacuum filtered through a 0.22 μm membrane (Millipore® Stericup and Steritop). The filtered liquid was eliminated; 2 g of the material deposited on the membrane surface was transferred in a 15 ml test tube and diluted with sterile Brain Heart Infusion broth to a final volume of 5 ml and homogenized by the vortex for 10–15 s.

2.4. Microbiological assessment

100 μl of each sample were seeded on the following media:

Chocolate Polivitex (PVX) agar, Columbia CNA agar with 5% of sheep blood (CNA), Mac-Conkey agar (MCK), Sabouraud agar (SAB), Mannitol Salt agar (MAN).

The CNA, MCK and MAN agars plates were incubated in aerobiosis at 37 °C for 24 h; the SAB Agar plates were incubated in aerobiosis at 30 °C for 24–48 h; one PVX agar plate was incubated in microaerophilia at 37 °C for 24–48 h and an additional PVX agar plate was incubated in anaerobiosis at 37 °C for 48–72 h.

2.4.1. Bacterial identification

The bacterial strains developed in aerobiosis or in microaerophilia were identified by GN1341 for Enterobacteriaceae and GP21342 for Gram-positive microorganisms. Reading of biochemical tests was obtained with the automatic reader (Vitek® 2 system Biomerieux).

The bacterial strains developed in anaerobiosis were identified by RAPID ID 32 A test (Biomerieux®), a standardized system with 29 miniaturized enzymatic tests. The test reading was carried out with (ATB® Expression®) software 4 h after anaerobiosis incubation at 37 °C.

Yeast was identified by ID 32 C test (Biomerieux®), a standardized system containing 32 miniaturized assimilation tests. The test