

New method for bacterial load measurement during hydrodebridement of cutaneous ulcer



Ann. Ital. Chir., 2008; 79: 187-192

Marco Apperti**, Adriana Di Lucia*, Luigi Goffredi*, Pasquale Federico***, Eddy Mattera***, Andrea Masella*, Mattia Davide Della Rocca**, Sarah Apperti**

Seconda Università degli Studi di Napoli, Dipartimento di Scienze Anestesiologiche, Chirurgiche e dell'Emergenza

* Servizio di emergenze chirurgiche

** Sezione di Flebologia e Studio della Riparazione Tessutale

*** Servizio di Angiologia

New method for bacterial load measurement during hydrodebridement of cutaneous ulcer

AIM OF THE STUDY: *Based on their experience using Versajet® for debridement of chronic wounds, the Authors set up a study protocol to verify whether the hydrosurgical cleansing could offer the possibility of taking tissue specimens suitable for diagnostic microbiological evaluation.*

The aims of the study were the following:

MAIN PURPOSE: *To evaluate the efficacy of hydrosurgery in detecting the presence of microorganisms and measuring their load, as an alternative to conventional tissue sampling methods;*

SECONDARY PURPOSE: *To set up an easier and less invasive diagnostic modality than surgical biopsy, even though likewise significant.*

RESULTS: *The results of this study show that tissue specimen collection by hydro-aspiration using Versajet® is comparable to biopsy sampling (and in some cases it can be even more reliable); moreover, it is not more time-consuming and is certainly less invasive.*

Compared to surgical biopsy, with such a method a greater amount of tissue may be collected; moreover, tissue specimens can be taken from a broader surface or, depending on the needs, from a more focused area on the margin or at the bottom of the wound.

KEY WORDS: Bacterial burden management, Hydrosurgery, Versajet.

Introduction

Removal of necrotic or non-vital tissue and infection control are essential - and very often inseparable - steps in the broadest context of cutaneous ulcer management. Issues related to bacterial culture for qualitative and quantitative examination are still matter of discussion, and many aspects are yet to be unanimously defined. In fact, rather simple and non invasive procedures for sample collection, such as needle aspiration or swab test,

have certain limitations in that they provide only qualitative or semi-quantitative information, or with regard to one source of contamination or a superficial infection: therefore, their utility in diagnosing a deep infection is doubtful.

Surgical biopsy using a scalpel, or preferably, a 3-6 mm punch, is certainly a more reliable technique.

With this method the microbiological testing has a greater significance; however, it is an invasive technique whose execution requires a certain degree of skill, and it cannot be repeated if other tests are needed. Another limitation is the poor compliance of the patient. Moreover, the biopsy specimen is necessarily taken only from a limited area, which is deemed to be the most suitable based on clinical judgment, although it might not necessarily be the most infected one.

Pervenuto in Redazione Aprile 2007. Accettato per la pubblicazione Luglio 2007.

Per la corrispondenza: Prof. Marco Apperti, Piazza De Sivo 2, 81024 Maddaloni (Caserta).

Some of these reasons may explain why most clinicians believe that a diagnosis of chronic ulcer infection can only be made on the basis of clinical relevancies.

Therefore, the therapeutic choice often depends on clinical diagnosis of infection and is modulated on the basis of clinical signs of bacterial activity; as a consequence, the biopsy is regarded as useless or unnecessary, or it will not be taken into consideration unless a clinical diagnosis is made.

Lastly, more frequent debridement procedures (the so-called *maintenance debridement*) are often needed in order to repeatedly remove not only tissue debris but also biofilms and slough from the ulcer, since they may extend healing time: in these cases, the potential utility of microbiological testing may certainly be conditioned by the invasiveness of surgical biopsy.

Aim of the study

Based on these considerations and on our experience using Versajet® for debridement of chronic wounds, we set up a study protocol to verify whether the hydrosurgical cleansing could offer the possibility of taking tissue specimens suitable for diagnostic microbiological evaluation.

The aims of our study were the following:

Main purpose: to evaluate the efficacy of hydrosurgery in detecting the presence of microorganisms and measuring their load, as an alternative to conventional tissue sampling methods;

Secondary purpose: to set up an easier and less invasive diagnostic modality than surgical biopsy, even though likewise significant.

Materials and methods

The study was performed in two steps, on a total of 20 patients presenting with chronic leg ulcers. For the first step of the study 10 patients were enrolled from January to April 2006, while for the second one the remaining 10 patients were enrolled from April to September 2006.

In the first part of the study we verified the correspondence between microbiological analysis of tissue specimens collected by biopsy and hydro-aspiration with Versajet®;

In the second part of the study we set up a method of tissue sampling that could be performed during debridement without hampering, nor lengthening, the execution of this latter.

In order to compare the two sampling techniques, for all wounds the bacteriological investigation was performed on tissue specimens taken by surgical biopsy and by hydro-aspiration of the effluent liquid collected during debridement.

PART I STUDY

Analysis on a group of 10 patients of the correspondence between microbiological analysis of tissue specimens taken by biopsy and hydro-aspiration with Versajet®.

Methods

Patient characteristics were reported on a dedicated form [Main pathology - Date of diagnosis - Previous antibiotic therapy (topical, systemic) - Date of samples (biopsy material, effluent fluid) - Concomitant pathologies]. All wounds were evaluated and documented using photography and millimetric scale (see Fig. 1).



Fig. 1: Reference (millimetric) scale for wound size assessment.

Any systemic or local antibiotic therapy was interrupted. Then, after a superficial cleansing of the wound using Versajet® with power level set to 3, a surgical biopsy was performed using a 5-6 mm punch.

In the first 6 patients, 3 specimens were collected by hydro-aspiration during ulcer debridement, as follows:

- the first one at the beginning of the debridement, in order to assess the presence of superficial contamination: the power of device was set to 5 and 100ml of fluid were collected from a 20cm²-surface;
- the second specimen was taken in order to detect any deep infection: the power of device was set to 7 or greater, and a volume of 250ml of fluid was collected starting from a 15cm-length of wound margins and then from a 20 cm²-surface of the central area;
- the third specimen was taken at the end of debridement, as a control, in order to standardize the method in relation to times and procedures: the power of device was set to the maximum level (9-10) tolerated by the patient and 250ml of fluid were collected with the same procedure as described above for collecting the second specimen.

In the remaining 4 patients only one 500ml-sample of fluid was collected during wound debridement, using the device with power set to the most suitable level that could be tolerated by the patient.

The biopsy tissues were analyzed according to the guidelines issued by "Standards Unit, Evaluations and Standards Laboratory - Specialist and Reference Microbiology Division".

The hydro-aspirated fluids were analyzed according to a procedure which consisted in tissue debris and effluent fluid separation and subsequent incubation of a known quantity of tissue.

Results

In this part of the study we obtained the following results:

- 1) In 4 patients with clear clinical evidences of an infected ulcer, the microbiological data from specimens collected by biopsy and hydro-aspiration were comparable (*Staphylococcus haemolyticus*, *Streptococcus equisimilis*, *Streptococcus agalactiae*, *Corynebacterium striatum*, *Enterococcus faecalis*).
- 2) In 3 out of 4 patients in whom the clinical diagnosis of wound infection was uncertain, microbiological data obtained with the two methods were comparable. In the remaining patient, who had an ulcer covered with a large necrotic eschar (see Figure 2), the analysis of biopsy specimen indicated that the Gram- were absent, while the one of the hydro-aspirated specimen detected the presence of *Pseudomonas aeruginosa* (110.000 CFU/gr of tissue). However, the greatest discrepancy was found between Gram+ quantification on the two sampling methods: in fact, the colony count of *Staphylococcus haemolyticus* was equal to 220.000 CFU/gr according to biopsy specimen analysis, while according to hydro-aspirated specimen analysis the count was greater than 10^6 CFU/gr.



Fig. 2: Necrotic eschar.

- 3) In one of two patients in whom a critical colonization was suspected, the two methods gave comparable results; in the other patient, presenting with large loss of substance of deep structures around the wound (see Fig. 3), the results on *Staphylococcus Haemolyticus* were remarkably different, since 54.000 CFU/gr of this

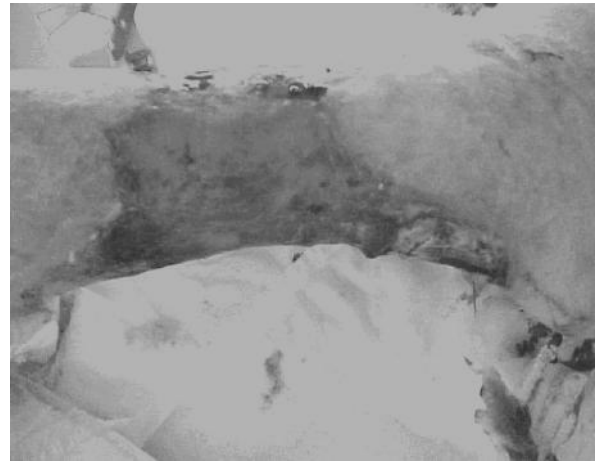


Fig. 3: Large loss of substance and deep structures exposition.

pathogen were detected according to biopsy specimen analysis, while according to hydro-aspirated specimen analysis the quantity was greater than 10^6 CFU/gr.

We can conclude that the two sampling methods gave comparable qualitative and quantitative microbiological results; specifically, surgical biopsy was comparable with microbiological testing of both single and multiple hydro-aspiration samples.

PART II

Set up of a method that allows tissue debris separation from effluent fluid at the same time of hydrodebridement, without disturbing or lengthening its execution.

Methods

We used a two-chamber sterile container. The upper chamber was connected to the Versajet® waste draining tube for collection of the effluent fluid, and the lower chamber was connected to a surgical aspirator (see Figure 4).



Fig. 4: Set for separation by filtration of debris tissues for microbiological testing.

In order to separate tissue debris from draining fluid, a filter (endowed with a prefilter, in order to avoid any obstruction) was positioned between the two chambers. We used either PS (polyethylene sulfone) or SFCA (cellulose acetate) membrane filter with a 0,45 μ m pore size, as they were the most adequate due to poor protein binding and high flow capacity.

Thanks to the filter, separation of tissue debris from fluid occurred at the very same time as collecting the effluent, so we could send the material directly for microbiological testing with no further handlings.

As a control, in all 10 patients a surgical biopsy was also performed.

Similarly to the first part of the study, we collected by hydro-aspiration 3 specimens in the first 6 patients and only one specimen in the remaining 4 subjects.

Results

The results of this part of the study showed that this new system allowed to obtain data comparable to those obtained during the first part of the study, with relation to both surgical biopsy and hydro-aspirated specimen analysis where tissue debris were separated in laboratory after effluent collection.

Moreover, we noticed that the specimen was suitable for cultural examination as soon as a sufficient quantity of filtered tissue could be collected, thus avoiding the filtration of a large amount of liquid.

Results and conclusions

The results of this study show that tissue specimen collection by hydro-aspiration using Versajet® is comparable to biopsy sampling (and in some cases it can be even more reliable); moreover, it is not more time-consuming and is certainly less invasive.

Compared to surgical biopsy, with such a method a greater amount of tissue may be collected; moreover, tissue specimens can be taken from a broader surface or, depending on the needs, from a more focused area on the margin or at the bottom of the wound.

Furthermore, this new method allows to collect one or more specimens from different areas, thus leaving the opportunity for sending all the different specimens for distinct microbiological analysis or combining the material for a single test.

As the procedure modalities are not unalterable and can be tailored to the needs from time to time, it thus follows that it is a very flexible system, easily adaptable to different situations.

Thanks to the ease of the procedure, microbiological analysis may be carried out not only in the presence of clear clinical signs of infection, but it could also become a method for confirmation of suspected infection; in other words, it could be practiced at any time deemed necessary. Unlike surgical biopsy, this procedure actually makes performing microbiological testing a pure clinical or experimental-decision making, which is not longer influenced by the limitations of the method.

Moreover, this method permits sampling at close intervals, thus allowing to evaluate the evolution of the ulcer in relation to the bacterial burden and with regard to the debridement itself.

Interestingly, all these advantages can be secured without hampering the execution of debridement procedure nor making the microbiological testing more difficult.

These results raised some questions that, rather than twisting or denying the value of our procedures, may ameliorate or act as a starting point for new experiences. In summary, our considerations were the following:

Is a microbiological testing actually needed only in the presence of clinical signs of deep infection?

Is a microbiological diagnosis necessary, other than appropriate, in case of critical colonization (superficial infection)?

Wouldn't be more appropriate to combine the indispensable cleansing of a non healing ulcer covered by a biofilm with a microbiological diagnosis?

For managing infection control, would it be therefore assumable the overcoming of today's clinical practice consisting in the initial clinical diagnosis of infection, followed by microbiological confirmation and, if needed, antibiotic therapy (see Table I).

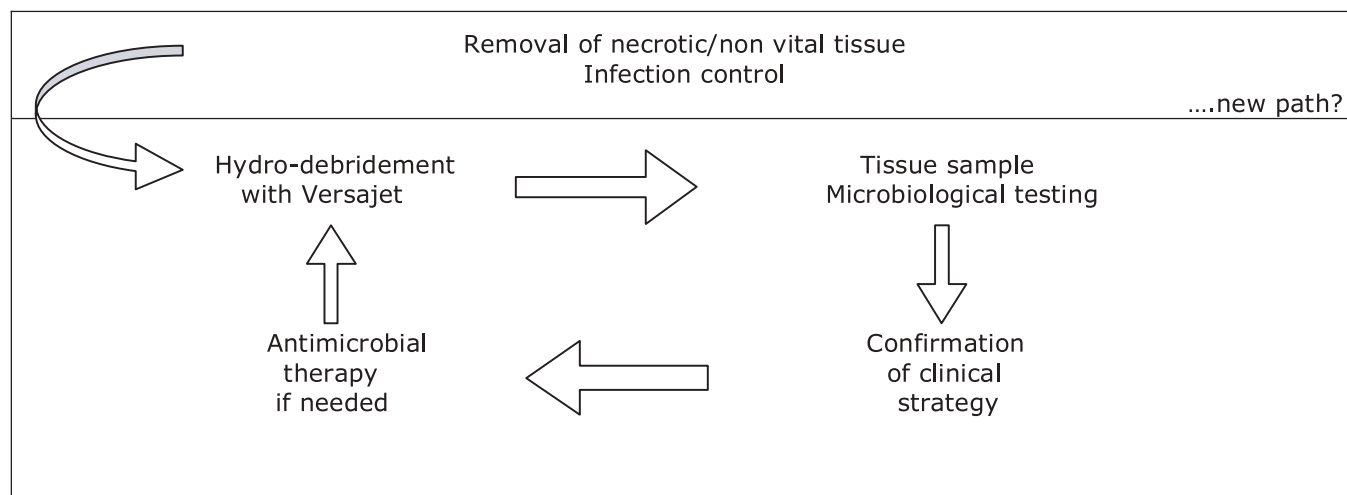
TABLE I - From clinical diagnosis to therapy

Clinical diagnosis	1) Colonization/Biofilm 2) Critical colonization/ Superficial infection 3) Deep infection	Cleansing + ++ +++
Microbiological testing	1) Useless ? 2) Appropriate 3) Necessary	
Topical antimicrobial therapy	Yes/No	
Systemic antimicrobial therapy	Yes/No	

In the actual context the debridement certainly plays a very important role, though is absolutely collateral. Instead, it could be taken into consideration an alternative clinical path in which the debridement procedure is regarded as the first therapeutic/diagnostic step, thus

allowing to remove tissue debris and reduce the bacterial load while simultaneously making the diagnosis of infection using simple and reliable quali-quantitative methods (see Table II).

TABLE II - Hypothesis of a new clinical path combining debridement with infection control



Riassunto

OBIETTIVI: Lo scopo dello studio è stato quello di mettere a punto una metodica originale per il prelievo da un'ulcera cutanea di un campione di tessuto da utilizzare a scopo colturale, con la stessa affidabilità della biopsia chirurgica ma con minore invasività e semplicità di esecuzione, da effettuare durante il debridement idro-chirurgico.

MATERIALI E METODI: Lo studio è stato condotto in due fasi ed è stato effettuato su 20 pazienti affetti da ulcere croniche degli arti inferiori, divisi in due gruppi. La prima fase è consistita nell'accertare la corrispondenza dell'esame microbiologico tra biopsia e prelievo con Versajet®. La seconda fase, invece, è servita a mettere a punto una procedura da eseguire durante lo stesso debridement, senza alterarne la modalità e senza allungarne i tempi. L'indagine batteriologica è stata effettuata sia su *campioni* biotici effettuati con punch da 5-6 min. che sul liquido refluo della lesione cutanea, in modo da poter confrontare le due tecniche colturali.

RISULTATI I FASE: Nel gruppo di 10 pazienti della prima fase abbiamo avuto i seguenti risultati: nei 4 pazienti in cui è stata posta diagnosi clinica di ulcera infetta, il dato microbiologico ha confermato l'orientamento clinico, con risultati colturali sovrapponibili nel campione biotico e nel tessuto prelevato durante il debridement.

In 4 pazienti con infezione dubbia abbiamo riscontrato in 3 di essi risultati sovrapponibili, mentre in un paziente con ulcera ricoperta da escara necrotica (Fig. 2) si è evidenziata notevole differenza di carica batterica riguar-

do ai Gram+ (*Staphylococcus haemolyticus*): nella biopsia vi è stato uno sviluppo di 220.000 UFC/gr. mentre nell'*hidroaspirato* è stata rilevata una carica superiore a 10^6 UFC/gr.

In 2 casi con sospetta colonizzazione critica abbiamo avuto in un paziente risultati sovrapponibili, mentre nell'altro, affetto da un'ampia perdita di sostanza fino alle strutture profonde (Fig. 3), vi è stata una notevole differenza di sviluppo di *Stafilococcus Haemolyticus* che ha dato 54.000 UFC/gr nella biopsia, mentre è stata superiore a 10^6 UFC/gr nell'*hidroaspirato*.

RISULTATI II FASE: Questa fase dello studio, relativa ad un gruppo anch'esso di 10 pazienti, è stata condotta nell'intento di mettere a punto una procedura intesa a separare i detriti tissutali dal liquido refluo raccolti durante il debridement idrochirurgico. Abbiamo usato per questo scopo dei contenitori sterili (Fig. 4) dotati di filtri.

L'analisi dei dati ottenuti dalla seconda fase ci ha dimostrato che con il dispositivo messo a punto, si ottengono risultati sovrapponibili sia rispetto alla prima fase, sia nei confronti della biopsia chirurgica.

Inoltre abbiamo potuto constatare come il prelievo divenga idoneo per l'esame colturale appena la quantità di tessuto filtrato è sufficiente ad essere sottoposta allo studio microbiologico, per cui non si rende necessario filtrare grandi quantità di liquido.

CONCLUSIONI: I risultati dello studio microbiologico hanno mostrato che il prelievo tissutale con Versajet è sovrapponibile alla biopsia.

Con tale metodica si riesce a prelevare una quantità di

tessuto non solo maggiore di quanto non si riesca con la biopsia chirurgica, ma il prelievo può essere eseguito, in funzione delle necessità, su una zona più ampia, oppure mirato ad una zona limitata, sul bordo o sul fondo della lesione.

Si è dimostrato, quindi, uno strumento flessibile ed adattabile con facilità a situazioni diverse, potendo variare agevolmente le modalità della procedura. La sua semplicità, fa sì che lo studio microbiologico delle ulcere possa essere eseguito non solo in presenza di evidenti segni clinici di infezione, ma anche in presenza di segni non univoci.

L'opportunità di eseguire un prelievo a scopo colturale, quindi, diviene condizionata solo dall'indirizzo clinico o sperimentale, e non da considerazioni legate ai limiti della metodica come può avvenire con la biopsia chirurgica.

Il nostro lavoro ci ha portato ad ipotizzare un superamento del percorso attualmente in uso: che parte dalla diagnosi clinica di infezione, attende conferma dell'esame colturale ed instaura una terapia antibiotica, (Tab. I) facendo sì che il debridement assuma un ruolo solo collaterale.

Potrebbe essere suggerito, invece, un nuovo percorso che preveda il debridement come primo atto terapeutico-diagnostico, potendo in tal modo ottenere contemporaneamente il risultato di allontanare i detriti tissutali, di abbattere la carica batterica e di porre diagnosi di infezione con specificazione quali-quantitativa semplice ed affidabile (Tab. II).

References

- 1) Falanga V: *Wound healing and its impairment in the diabetic foot*. Lancet, 2005; 366:1736-743.
- 2) Apperti M: *Il ritardo della riparazione tissutale cutanea: alla scoperta delle cause: Infezioni*. In: *Ulcerazioni e cicatrizzazione cutanea. Nuovi concetti, nuove terapie*. Torino: UTET, 2004.
- 3) Falanga V, Margolis D, Alvarez O, et al: *Rapid healing of venous ulcers and lack of clinical rejection with an allogenic cultured human skin equivalent*. Arch Dermatol, 1998; 134:293-300.
- 4) Apperti M, et al: *Nuova metodica per lo studio della carica batterica durante hidro-debridement delle ulcere cutanee*. Atti Giornate Scientifiche di Facoltà. Napoli, Italy, June 2006.
- 5) Robson MC: *Wound infections, a failure of wound healing caused by an imbalance of bacteria*. Surg Clin North Am, 1997; 77(3):637-50.
- 6) Drosou A, Falabella A, Kirsner RS: *Antiseptic on wounds: An area of controversy*. Wounds, 2003; 15(5):149-66.
- 7) Apperti M, et al: *Sorveglianza Epidemiologica dei Trattamenti delle Infezioni Gravi (SETIG): risultati principali e di metodo*. Giornale Italiano di Farmacologia Clinica, 1997; 11(4):269-300.
- 8) Tarnuzzer RW, Schultz GS: *Biochemical analysis of acute and chronic wound environments*. Wound Rep Regen, 1996; 4(3):321-25.
- 9) Dang C, et al: *Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: A worsening problem*. Diabet Med, 2003; 20:159-61.
- 10) Masella, M. Apperti, I. Verde, et al: *L'argento metallico catalitico nella terapia topica del paziente ustionato*. In: *Le ustioni, argomenti di clinica e terapia*. Napoli: De Nicola ed. 1999: 121-26.
- 11) Falanga V: *FACP Classifications for wound bed preparation and stimulation of chronic wounds* Wound Rep Regen, 2000; 8:347-52.
- 12) Apperti M, et al.: *Nosocomial Infections: Which patients do we need to Survey?* 14° International Conference on Pharmacoepidemiology. Berlin, Germany, August 16-19, 1998.
- 13) Canonico S, et al: *Uso topico dei "fattori di crescita" nel trattamento delle ulcere flebostatiche "difficili" degli arti inferiori*. Acta from 6° Congr. Naz. C.I.F. Lecce, Italy, October 3-6, 2002.
- 14) D'Andrea F, Vitagliano T, Nicoletti G: *Le ulcere flebostatiche: trapianto autologo*. In : *Flebologia 2001*. Napoli: Ed De Nicola, 2001.
- 15) Piaggese A, Baccetti F, Rizzo L, Romanelli M, Navalesi R, Benzi L: *Sodium carboxyl-methyl-cellulose dressing in the management of deep ulceration of diabetic foot*. Diabet Med, 2001; 18:320-24.