Modern Medicine. 2015, Vol. 22, No. 4 : 375-381

Review

Controversies and challenges of chronic wound infection diagnosis and treatment

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REZUMAT

Controverse și provocări în diagnosticul și tratamentul infecțiilor de la nivelul plăgilor cronice

Pe parcursul ultimului deceniu, plăgile cronice, precum ulcerele venoase sau arteriale, ulcerele diabetice, escarele și plăgile chirurgicale cu vindecare întârziată, au captat atenția comunității medicale, datorită prevalenței în creștere și a costurilor ridicate de tratament. Insuficiența vasculară reprezintă principala cauză de apariție a ulcerațiilor cronice, în timp ce infecția este cea mai frecventă complicație. Infecțiile cronice persistă și progresează în ciuda unui tratament antimicrobian adecvat și sunt de obicei cauzate de biofilme mono- sau polimicrobiene. Colonizarea bacteriană persistentă a plăgilor, precum și utilizarea pe termen îndelungat a antibioticelor predispun spre dezvoltarea infecțiilor nosocomiale cu tulpini rezistente, situație în care complicațiile septice implică un risc vital, în special la indivizii imunocompromiși. În acest articol, efectuăm o revizuire aprofundată a literaturii științifice, pentru a răspunde principalelor controverse privind implicarea microorganismelor planctonice și/sau biofilmelor în procesul de vindecare a plăgilor cronice. De asemenea, ne propunem să analizăm utilitatea tratamentului antimicrobian în ulcerele cu vindecare întarziată și să stabilim principalele sale obiective, spre a obține beneficiul terapeutic maximal.

Cuvinte cheie: infecție cronică, plagă cronică, biofilm, antibiotic, antiseptic

ABSTRACT

Over the last decade, chronic wounds such as venous or arterial ulcers, diabetic foot ulcers, pressure sores, and non-healing surgical wounds were brought into the spotlight of the medical community, due to their increasing prevalence and to their significant economic burden. Vascular impairment represents the main cause of chronic ulceration, while the infection is the most frequent complication. Chronic infections persist and progress despite an adequate antimicrobial regimen and are typically caused by mono- or polymicrobial biofilms. The persistent bacterial colonization of the wound, as well as the longterm use of antibiotics predispose to the development of nosocomial infections with resistant strains, with the risk of life-threatening septic complications, especially in immunocompromised individuals. In this article, we perform a thorough literature review, in order to answer the main controversies regarding the involvement of planktonic

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and/or biofilm bacteria in the healing process of chronic wounds. Furthermore, we aim to analyse the utility of antimicrobial treatment in non-healing wounds, and to establish its main end-points, for the optimal benefit of the patients.

Key words: chronic infection, chronic wound, biofilm, antibiotic therapy, antiseptic

INTRODUCTION

Over the last decade, chronic wounds such as venous or arterial ulcers, diabetic foot ulcers, pressure sores, and non-healing surgical wounds were brought into the spotlight of the medical community, due to their increasing prevalence and to their significant economic burden (1). In developed countries billions of dolars are spent each year (2,3) for the repeated hospitalizations and expensive treatment of patients suffering from non-healing ulcers. The persistent pain, either spontaneous or induced by treatment (4), the malodour of the ulceration (5), the mobility restrictions (6), and the excessive exudate, significantly impair the patients' quality of life, who might also experience secondary mood disorders (50-75%) (6) or sleep disorders (69%) (7). The persistent bacterial colonization of the wound, as well as the longterm use of antibiotics predispose to the development of nosocomial infections with resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamases (ESBLs) producing microorganisms, and multiple antibiotic resistant *Pseudomonas aeruginosa* (8). Immunocompromised individuals, either institutionalized or hospitalized, are particularly affected by the chronic infection with resistant strains, due to their higher risk of developing life-threatening septic complications (9). Other severe outcomes are represented by limb loss (10) or malignancy (5).

Chronic or refractory wounds are defined as lesions that don't show any tendency to heal after 3 months of standard therapeutic care or that still persist after 12 months of appropriate treatment (11). Chronic infections persist and progress despite an adequate antimicrobial regimen (12) and are typically caused by mono- or polymicrobial biofilms. This theory aims to explain the pathogenic role of bacteria in the non-healing trajectory of chronic wounds. Although several recent studies associated wound infection with the process of delayed healing, controversy still exists, since current literature is equivocal on the subject (13-15). However, antimicrobial therapy remains the mainstay of treatment.

In this article, we perform a thorough literature review, in an attempt to answer the main controversies regarding the involvement of planktonic and/or biofilm bacteria in the healing process of chronic wounds. Furthermore, we aim to analyse the utility of antimicrobial treatment in non-healing wounds, and to establish its main end-points, for the optimal benefit of the patients.

Chronic wound infection

Wound bacteria impede normal healing?

In recent scientific literature, the microorganisms most frequently identified by traditional culturing techniques from various types of chronic wound samples, were represented by species of *Staphylococcus* (47%-55%), mainly *S. aureus* and *S. epidermidis* (16-18), by *P. aeruginosa* (25%, 33.6%) (17,18), *Enterococcus faecalis* and *Enterobacteriaceae spp.* such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter spp.* (8,16). Some reports warn on the increasing incidence of multiresistant Gram negative bacteria colonizing chronic skin ulcers (8,19). Several studies revealed by anaerobic culturing techniques or by molecular microbial diagnostic tests (Polymerase Chain Reaction, 16S DNA sequencing), the high proportion of anaerobic bacteria of the wound microbiota (20-22). Most often, wounds are colonised by multiple microbial species, bacteria and fungi, aerobic and anaerobic, and, therefore, it is very difficult or even impossible to make a prediction of their individual impact on wound-healing (23).

Previous data suggets the implication of biofilm bacteria in approximately 80% of all human infections (24,25), while in chronic wounds the presence of mono- or polymicrobial biofilms was documented in about 60% of cases (26). Recently, it was argued that biofilms exist in all chronic wounds, due to the general property of microorganisms to attach to a surface and, consequently, to the wound base, where they develop complex communities (14).

When environmental factors become unfavourable for the optimal development and multiplication of free-floating bacteria, the microorganisms switch into a biofilm phenotype as a survival strategy (27). Clusters of aggregated cells, adhered to a surface or floating free in organic matter (28), are embedded in an extracellular matrix, with mixed origin, both self-produced (polysaccharides, proteins, DNA, RNA, lipids) as well as originating from the host (DNA, RNA, fibrin, platelets, immunoglobulins) (12,29).

The biofilm is characterised by tolerance to antimicrobial therapy, gained through various mechanisms such as the variability of baterial methabolic rates from the surface to the centre of the biofilm (30), by the action of antibiotic degrading enzymes (30), or the mechanical obstacle represented by the extracellular matrix (31). This fact explains why antibiogram-guided antibiotherapy may

be associated with treatment failure and infectious relapses (12). Moreover, the mono- or polymicrobial biofilms display tolerance to the host's immune defense mechanisms, primarily through resistance to immune cell phagocytosis (16, 32, 33), while they trigger a persistent, low-intensity inflammatory response, different from the exuberant systemic response of planktonic bacteria (12, 34).

It is rational that both bacterial phenotypes, planktonic or biofilm, interfere with the healing of acute and chronic wounds, since they already have assigned roles in the occurrence of exacerbations or in the maintenance of a persistent inflammatory status (14). This is especially true when clinical signs of infection are present. Group A and G beta-haemolytic streptococci, *Pseudomonas aeruginosa* were associated with an unfavourable outcome (35). On the other hand, Kostarnoy AV et al. (2013) observed an improved healing rate after topical application of bacterial lipopolysaccharide in acute wounds (36), while Kanno E et al. (2013) noticed that low levels of bacterial contamination might enhance tisular regeneration (37). The difference between comensal and pathogenic biofilm bacteria should be influenced by the virulence of the microorganisms.

Few authors studied the virulence pattern of bacteria and their ability to form biofilms. In patients diagnosed with venous ulcers, arterial ulcers, sacral pressure sores, thoracic wounds secondary to breast cancer, non-healing surgical wounds, and abscesses, Mihai MM et al. (2014) revealed that all of the studied *P. aeruginosa* strains intensely developed biofilms (**Fig. 1**), followed by *S. aureus* strains which had low differences between methicillin and non-methicillin resistant bacteria, while Enterobacteriaceae spp. showed a low capacity to form such structures at 24, 48 and 72 hours of incubation (8).

We support the idea of Gottrup F et al. (2014) that not all wound bacteria should be removed, although a clear causal or protective role of microorganisms in wound healing has not yet been established (35).

Should we regularly include aerobic culturing in the diagnosis of chronic wound infection?

Depending on the availability of several laboratory tests in wound care facilities, the following clinical and laboratory features may be considered useful in biofilm diagnosis: the presence of clinical signs of infection, especially if they lasted more than 7 days, the failure of antibiotic treatment and reccurence of infection, the reappearance of systemic signs and symptoms of infection after antibiotherapy cessation, the detection of mucoid *P. aeruginosa* (12, 38), a biofilm-specific microbial phenotype, the microscopic evidence of microbial aggregates and biofim structure, surrounded by inflammatory infiltrates, positive molecular diagnostic methods (Polymerase Chain Reaction, Fluorescence in situ Hybridization, Pyrosequencing or Next-generation Sequencing) and even a specific immune response to the identified microorganisms, expected after 2 weeks of biofilm infection (12).

In the diagnosis of chronic wound biofilm infection, the utility of aerobic culturing techniques of superficially collected samples was repeatedly questioned because they may yield false-positive results in case of contamination with skin microflora. Also, false negative results can appear despite suggestive clinical signs of infection, due to an increased adherence of biofilm bacteria to the surrounding tissues, to the presence of non-culturable bateria, with low metabolic rates, and to the impossibility to diagnose anaerobic microorganisms (9, 12, 39). Moreover, traditional culturing cannot differentiate between planktonic or biofilm bacteria.

The most recent guideline of biofilm diagnosis recommends, in case of severe or moderate soft tissue infection, to perform a tisular biopsy from the base of the debrided wound, followed by histopathological examination. Although this reliable technique can objectivate the microbial aggregates, as well as the accumulation of polymorphous nuclear cells (PMNs) at the infection site (40, 41), it cannot identify the causative microorganisms and contribute to the therapeutic approach (9, 42).

Formerly considered the gold standard of wound infection diagnosis, aerobic culturing may still provide useful information when it is associated with antibiotic susceptibility testing in order to guide antibiotherapy (12), especially for biofilm dispersed bacteria and risk of systemic infection. The results of antibiotic susceptibility

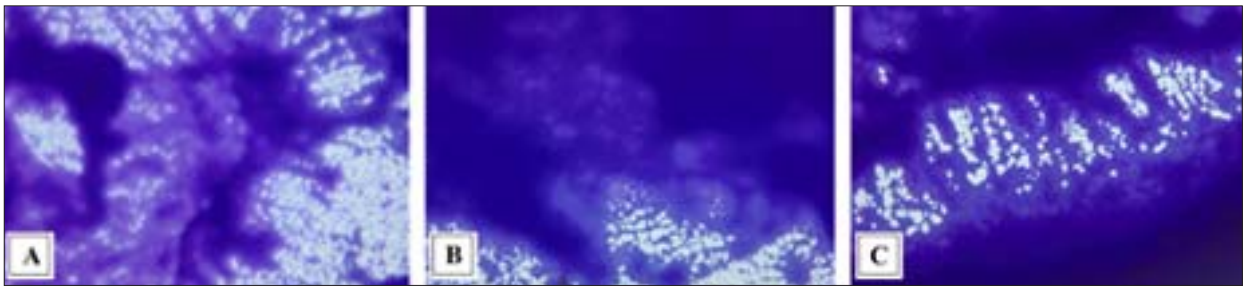

Figure 1. Microscopy analysis of *Pseudomonas aeruginosa* biofilms developed after 24 hours (A), 48 hours (B) and 72 hours (C) (Personal library, CDPC, Colentina Clinical Hospital, Bucharest; Department of Microbiology, Faculty of Biology, University of Bucharest)

testing can, however, be misleading in biofilm infections, due to their characteristic tolerance to such therapeutic agents. The Calgary Biofilm Device was designed to detect in a standardised, repeatable, and reproducible method the minimum biofilm eliminating/eradication concentration (MBEC) (43, 44).

In acute infections, such as urinary tract infections, a quantitative microbiological assesment guides the therapeutic approach (the presence of more than 105 bacteria/mm^3). In non healing-wounds and chronic infections no clear relationship has been established yet between the bacterial load and the clinical signs of infection 35. Critical colonisation defines the ammount of bacteria present in non-infected, non-healing wounds, involved in the pathogenesis of the skin lesions.

What is the optimal treatment of chronic wound infection: antibiotics, antiseptics or other therapeutic agents?

In order to efficiently treat chronic infections, the approach should initially focus on the prevention of microbial attachment and biofilm development, followed by the selection of therapeutic agents with an increased delivery to their biofilm target (9). Moreover, to achieve optimal functional results, adverse reactions, such as allergy or intolerance, should be rapidly diagnosed. The emergence of resistant strains should be carefully assessed by periodic microbiological examination of wound samples, associated with antibiograms for the isolated bacteria (9).

If antimicrobial therapy is to be considered the ideal therapeutic approach in patients with chronic wounds, then it should accomplish the following: prevent and treat wound infection, promote wound healing and increase the patient's quality of life (45). Moreover, an ideal antimicrobial agent should remove all pathogenic bacteria, or at least reduce the bacterial load below the critical colonization limit, should spare the comensal microflora and should support the host's defence mechanisms (45).

While resistance to antibiotics is genetically acquired and therefore it is irreversible, the tolerance of the biofilm to antimicrobials may revert to susceptibility after a phenotypic change to a free-floating status (9). In chronic wound infection, standard antibiotic therapy alone cannot eradicate biofilm infection because the appropriate concentrations reach values 1,000 times higher compared to the ones for planktonic bacteria (46, 47), and cannot be used in clinical practice due to the associated toxicity. Moreover, due to hypermutability and an increased horizontal gene transfer, biofilm communities generate in higher rates resistant bacterial strains at higher rates (48, 49).

In cystic lung infection with *P. aeruginosa*, during the phenotypic change, it was documented the existence of a so-called "window of opportunity" occurs, which signifies a period of microbial vulnerability to an aggressive antibiotic therapy (50). This might suggest the utility of an antibiotic pulse-therapy, repeated periodically, in order to maintain the bacterial load at unharmful levels as pre-emptive treatment. However, there is no evidence to support the use of systemic antibiotics to prevent or to treat biofilm wound infection despite their common use in clinical practice (12, 51-53).

No evidence is available regarding the optimal therapeutic approach in chronic wound infections with mature biofilms, the context in which topical antimicrobials should be initiated (non-healing non-infected wound vs wounds with clinical signs of infection) or the exact antimicrobial targets (wound sterilization vs the sparing of "good bacteria", fungi or bacteria) (12). Høiby N et al. suggested that combined therapy is a better option (two different classes of antibiotics, local and systemic therapy, local antibiotic and local antiseptic) (12). Also, the application of antimicrobials on a previously debrided wound should be effective (12, 54), because it would remove residual free-floating bacteria, the main source of biofilm restoration (35).

Therapeutic decisions should not be based on the price of a single product, but rather on a thorough evaluation of the full costs of treatment, with the premises that it would take place on a long period of time and it should achieve wound healing (35). The overuse of antibiotics, a main cause of the rising prevalence of microbial resistance, should be prevented by the education of both patients and healthcare practitioners (55).

The efficacy of antimicrobial therapy is optimally assessed by objective clinical measures of wound progression, using standardised questionnaires such as the Wound Healing Index (56), as compared to the removal of microorganisms from ulcers (35).

The alternatives to antibiotic treatment

Since topical and systemic antibiotherapy have the previously mentioned downsides, antiseptics represent a promising alternative for the removal of wound bacteria. Topical antiseptics such as chlorhexidine, povidone iodine, hydrogen peroxide, boric acid, acetate, silver sulfadiazine or nitrate, and sodium hypochlorite have been widely used both as curative as well as a palliative treatment of chronic wounds, in order to prevent or to treat infections. Applied daily, they persist within the wound environment approximately 24 hours. However their beneficial effect on wound healing has been under debate in the last few years, due to their assumed citotoxicity, revealed by in vitro studies. Future studies should focus on their effects in vivo, quantified at variable concentrations (57). Daeshlein G (2013) assigned the most efficient and best tolerated antiseptics: octenidine dihydrochloride and polyhexanide (45), to which microbial resistance in vitro has not yet been reported (35). However the antiseptic which mostly promotes wound healing is assumed to be polyhexamethylene biguanide (polyhexanide) (50).

Intuitively useful, a thorough wound debridement, either mechanical, enzymatic or biological, is the recommended approach of some authors, although, it has a temporary effect as single therapy and might even promote the inoculation of infection in deeper tissues (59).

The patient's general health status- an obstacle to overcome

Patients diagnosed with non-healing ulcers frequently associate predisposing factors such as sedentary lifestyle, alcohol consumption, older age or suffer from associated comorbidities, such as nutritional disorders (obesity, diabetes), cardio-vascular diseases (arterial hypertension, atherosclerosis), suggesting the need of a multidisciplinary approach (1). Daeshlein G (2013) pointed out the interdependence between the patient's general health status and wound healing, because each of them benefit from the amelioration of the other one (45).

The treatment of underlying respiratory or circulatory diseases, associated with tisular oxygen deprivation, as well as the implementation of health supporting measures, such as an adequate maintenance of gaseous exchange or weight control, play critical roles in an efficient wound care approach (45). The haemodynamic health in venous ulcerations might be achieved by vascular sclerotherapy or stripping, intraluminal ablative intervention (laser and steam), and the treatment of thrombotic syndrome, while in arterial wounds, vascular repermeabilisation by recanalisation, stent or bioprosthesis implantation, dilatation or pharmacological intervention with vasoactive components might promote wound healing (45).

When standard therapeutical measures fail, palliative care with multidisciplinary treatments should be initiated in order to achieve symptomatic improvement, as well as an enhancement of the quality of life of the patient and his family (60). It is also reasonable to consider novel and experimental therapeutic regimens, when all conventional treatment options have been exhausted (45).

CONCLUSIONS

The dermatologist often provides the continuing care of patients who have failed to achieve wound closure after medical and/or surgical treatment in departments of diabetology or vascular surgery, which involves creative thinking in the adaptation of treatment regimens to the unique needs of each patient, altogether with the control of other comorbidities. The early identification of high risk patients, less likely to respond to conventional therapies requires a solid and thorough knowledge of the etiopathogenic and aggravating factors that contribute to the non-healing course of chronic wounds.

The vascular impairment, the wound microbiota- with either planktonic or biofilm phenotypes, the local inflammatory response, as well as the comorbidities of the patient should be considered in the diagnosis and treatment of chronic wounds.

The relationship between the wound bacterial load and clinical signs of infection has not been established, and, therefore, the quantitative approach of microbiological diagnosis is not helpful, but rather the identification of the bacterial species and their virulence. Further studies should focus on establishing clear correlations between the virulence and resistance phenotypes of bacteria isolated from chronic wounds and the clinical picture. Also, a stricter definition of critical colonization is needed, while a clear causal or protective role of microorganisms in wound healing remains to be established.

Our recommendations of wound infection diagnosis and treatment differ depending on the observed clinical signs. When there are no signs of infection, in the so-called non-infected, non-healing wounds, aerobic culturing and systemic antibiotic therapy are not recommended, but we rather consider useful the pre-emptive approach that includes a thorough wound debridement and topical, well-tolerated antiseptics (**Fig. 2**). In case of severe or moderate soft tissue infection, aerobic culturing may still provide useful infor-

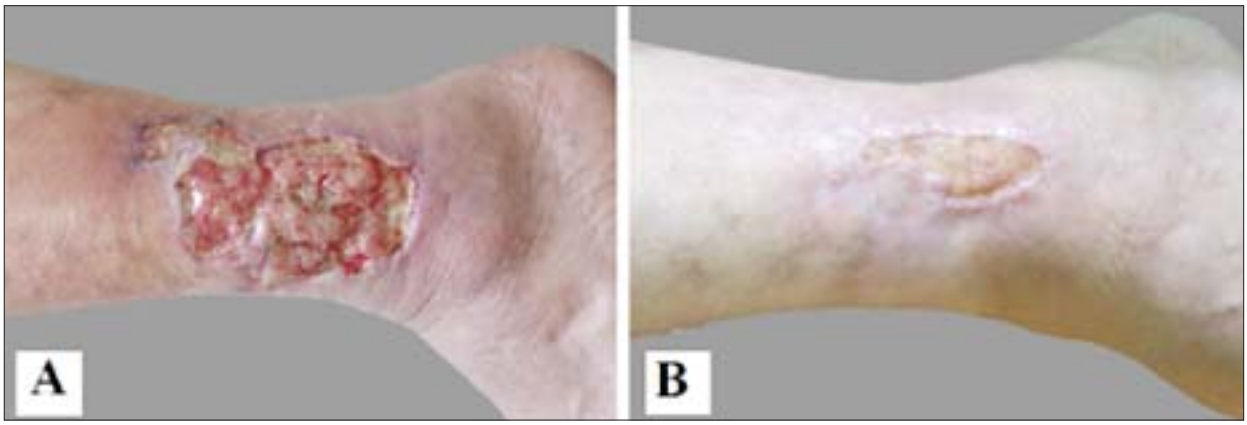

Figure 2. Wound progression A-Before treatment; B-6 months after personalised wound-care

mation when it is associated with antibiotic susceptibility testing in order to guide antibiotherapy. However, if possible, the most specific biofilm diagnostic procedure would be the histopathological examination of a superficial tisular of the debrided wound, enabling the clinician to adapt his approach towards biofilm-targeting therapeutic agents.

Although there is no evidence to support the use of systemic antibiotics to prevent or to treat biofilm wound infection, this approach is commonly used in clinical practice, giving rise to microbial resistance and nosocomial infections. Combined antimicrobial therapies might represent a better option, while the most recent guidelines of biofilm infection recommend the application of antiseptics such as octenidine dihydrochloride and polyhexanide oon previously debrided wounds. Future research should focus on the development of alternative antimicrobial treatments in non-healing wounds.

ACKNOWLEDGEMENT

This paper is partly supported by the Sectorial Operational Programme Human Resources Development (SOPHRD), financed by the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/141531.

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