Identifying infection in chronic wounds

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Abstract

There is a myriad of published research and anecdotal information available regarding wound infection, biofilm and antimicrobials. The author reviewed recent literature on chronic wound infections and has provided a concise and simple breakdown aimed at health professionals dealing with chronic wounds to encourage critical appraisal of their current practice and to guide future practice.

Keywords: chronic wound, infection, swab, biofilm, investigations.

Introduction

Intact skin is the perfect defence to bacterial invasion, but damage to the skin allows bacteria, fungi and yeasts to enter. More than 200 different species of bacteria normally live on the skin[1] and an open wound provides a moist, warm and nutritious environment perfect for microbial colonisation and proliferation. Bacteria colonise all chronic wounds and low levels of bacteria can benefit the wound by increasing the amount of neutrophils, monocytes and macrophages in the wound, thus improving levels of prostaglandin E2 and the formation of collagen[2]. When one or more microorganisms multiply in the wound, local and systemic responses occur in the host, which can lead to infection and a subsequent delay in healing[3]. Maintaining the bacteria at a level at which the host is in control is an important part of avoiding wound infection[4].

Regardless of large amounts of bacteria, many wounds continue to heal well. The ability of the patient's immune system to deal with bacteria (host response) and the type and amount of bacteria involved determines whether clinical problems will occur[5]. Chronic wounds are open for extended periods of time and the patients usually have underlying disease processes, which leads to heavy colonisation with bacteria and/or fungi[6]. When chronic wounds are poorly perfused they are more susceptible to infection, as blood delivers oxygen, nutrients and immune cells, thus providing little opportunity for microorganisms to colonise and proliferate[7]. Devitalised tissue, combined with fluid and nutrients from wound exudate provide an ideal setting for bacterial proliferation[4]. The host response can often be improved by correcting or improving the underlying diseases[6].

Repeated wound infections can lead to depression and anxiety for the patient due to increased systemic symptoms and an obvious visible deterioration of the wound[8]. They are expensive[5] and cannot heal, increasing treatment costs and the demand on nursing resources[7]. Therefore, efficient diagnosis and treatment of wound infection is essential. However, this can prove to be challenging and as there is no expert consensus on the best assessment methods, it is entirely dependent upon the skill of the individual clinician[9].

Clinical assessment

The terms used to identify infection can be very confusing. International consensus[5] suggests using the following definitions:

Contamination – bacteria within the wound but not causing clinical problems.

Colonisation – bacteria multiplying but no damage to wound tissues.

Infection (local) – bacteria multiplying, healing stalls and wound tissues are damaged.

Infection (spreading) – bacteria may cause problems close to the wound.

Infection (systemic) – bacteria spreading, causes systemic illness.

Clinicians need to be able to quickly and confidently identify and treat wound infections, to enable healing to commence[10].

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This entails recognising the early clinical changes in the inflammatory response[11]. In a new wound, the inflammatory response starts the healing process by activating the immune system. This process would normally last a few days, until infection is prevented by the proliferation of new cells and the wound becomes sealed. In chronic wounds, this process differs in that the inflammatory response lasts a lot longer, causing chronic inflammation and the inability to move through the stages of repair. This inflammation may be misdiagnosed as infection[10]. Acute wounds display the classic signs and symptoms of infection more than chronic wounds, in which the signs may differ according to aetiology. Immunologic or vascular impairment can mask infection, whilst necrotic tissue or foreign matter can increase it[12]. This makes interpretation of clinical signs very difficult[13].

Heat to the surrounding skin is one of the signs of infection[14]. Research has shown that the mean temperature difference between periwound skin and a control site was <2° in the absence of infection, and >2° when infected. However, before diagnosing clinical infection there should be at least two other indicators present[9].

In the presence of infection, there is often an increase in exudate, but this in itself is insufficient to diagnose infection. The colour, consistency and odour of the exudate are also important and may indicate components, contaminants or underlying causes[15]. Clear, amber exudate is usually considered 'normal', but may indicate infection by bacteria such as *Staphylococcus aureus*, or may be fluid from a urinary or lymphatic fistula. Green/blue exudate may be indicative of bacterial infection, such as *Pseudomonas aeruginosa*. Thick, sticky exudate may be due to high protein content from infection or inflammatory processes, or due to necrotic material, enteric fistula or residue from dressings or topical preparations. Unpleasant odour may be due to bacterial growth or infection, necrotic tissue, sinus or urinary fistula[15].

An interesting study by Miller *et al.*[13] revealed that there is very little relationship between the bacterial burden detected by a semi-quantitative swab and the clinicians' assessment of critical colonisation or infection. All wounds accepted to the study had one or more clinical signs of critical colonisation or infection (with an average of 3.3 signs), yet microbiology results showed that almost 40% had nil or scanty bacterial growth and about 66% had no leukocytes.

It has been suggested using the mnemonics "NERDS" and "STONES" to assist in differentiating between wounds with increased bacterial burden which may respond to topical antiseptics and those with deep infections that need systemic antibiotics[14] (Table 1).

Table 1. The mnemonics "NERDS" and "STONES" used to differentiate between wounds which may respond to topical antimicrobials, and those requiring systemic antimicrobials[14].

Superficial infection		Deep infection	
N	Non-healing wounds	S	Size – bigger
E	Exudating wounds	T	Temperature – increased
R	Red and bleeding granulation tissue	O	Os (probe to or exposed bone)
D	Debris on wound surface (yellow/black)	N	New or satellite areas of breakdown
S	Smell	E	Exudate, oedema, erythema
		S	Smell

Which laboratory test?

A diagnostic tool needs to be simple, quick and able to be used at the point of care; provide quantitative and/or qualitative information on a range of organisms; not be invasive; provide an accurate reflection of what is happening within the wound and any biofilm, and indicate when there is a need for intervention[16]. Two issues with many diagnostic tests are the delay in reporting and issues with sampling techniques[16].

Initial investigation of C-reactive protein and white blood cells will help differentiate between inflammation and infection[17]; whilst erythrocyte sedimentation rate and serial C-reactive protein levels are useful for monitoring response to antibiotics[18].

Microbiology tests should not be routine for all wounds, but restricted to situations when bacterial load is thought to be delaying healing[6]. Tissue biopsy is considered the gold standard for microbiology sampling but is expensive, time-consuming, invasive, painful and may disrupt any healing[3]. Surface swabbing is easier, cheaper and non-invasive[6] and results have been shown to compare favourably with results of quantitative biopsy[13]. Cooper *et al.*[19] agree that although a little extra information is gained from a biopsy than a swab, invasive tests cannot be justified to detect infection in chronic wounds without bone involvement. Molecular technology has recently been able to establish the presence of many microorganisms living in a viable but non-culturable state in chronic wounds. They have not previously been recognised but probably still had a significant effect on infection and non-healing[20]. Wolcott[21] reports that laboratory cultures often give variable results and that molecular diagnostics is a more accurate way of diagnosing bacteria within a biofilm. Polymerase chain reaction is used to screen samples for bacteria commonly found in wounds: *Staphylococcus,*

Methicillin-resistant *S. aureus*, *Enterococcus*, Vancomycin-resistant *Enterococcus*, *Streptococcus* A and B, *Pseudomonas aeruginosa*, *Candida albicans*, *Serratia*, *E. coli* and so on. Results are available the same day with a second test that produces a report confirming all the bacteria in the sample within four days. However, this is not currently common practice.

Surface swabs

The most commonly used clinical diagnostic tool is the wound swab[22]. Microbiology swabs should be used as an adjunct to clinical assessment and not as a first-line strategy for diagnosing infection. Culture can indicate the predominant flora in wounds, identify resistant organisms and suggest systemic treatment for infected wounds[18]. Many swabs are taken routinely but not supported by clinical need, and incorrect techniques are used when taking and transporting the specimens[1]. Swabs should be taken before commencement of antibiotic therapy as this can affect the results of the culture. They must be taken correctly to ensure they collect organisms from within the tissues rather than surface contaminants[3].

The wound should first be cleansed with normal saline or sterile water, to ensure samples do not represent surface bacteria and loose debris, but deeper microbiology[23]. Antiseptics should be avoided as they may alter the result[24]. If the wound is not cleansed first, the amount of microflora obtained will make the isolation and identification process more difficult. Should a swab be taken from a wound prior to thorough cleansing, the laboratory should be informed[1].

It is important to swab viable tissue rather than areas of slough or eschar and to avoid pockets of pooled exudate, which often contain surface contaminants rather than pathogens[24].

The "Z" method is sometimes suggested, which is swabbing in a zigzag pattern, or swabbing more than one area for larger ulcers[18]. However, a recent Australian study[3] illustrated that the Levine method of wound swabbing was more reliable in identifying the wound organisms than the "Z" method. The Levine method involves rolling the swab tip over 1 cm^2 of cleansed granulation tissue using enough pressure to obtain fluid from within the wound[23]. The increased reliability may be due to the fact that pressure applied to the wound bed releases planktonic bacteria from the biofilm, enhancing the sample collected[3].

When possible, tissue or pus, or both, should undergo quantitative microbiological analysis as growth from these samples will be representative of pathogenic flora[25]. If the wound is dry, then the swab may be pre-moistened in transport medium[23]. Be aware that classic signs and symptoms of bacterial burden may occur prior to being detectable by swabbing; these signs and symptoms may actually represent chronic inflammatory changes; and the presence of biofilm can create a physical barrier to accurate sampling[13].

Swab results

All wounds contain bacteria but many heal regardless. This is determined more by the host/bacterial interaction that the mere presence of bacteria[3]. Clinicians should remember when interpreting laboratory reports that the inclusion of antibiotic sensitivities does not mean that the wound is infected and that the presence of infection should be determined on the basis of clinical signs and symptoms and not the laboratory report in isolation[26].

Swab results should never overrule clinical judgement, but sometimes a swab result may be inconsistent with the clinical features. For example, a bright green exudate suggests *Pseudomonas* infection and appropriate antibiotics may be commenced even if the swab fails to culture *Pseudomonas*[27].

Many bacteria identified in human infections do not grow or grow poorly with commonly used agar-based cultivation methods[22]. *Staphylococcus aureus* survives this procedure extremely well so is often listed in laboratory reports. Anaerobes, however, do not grow well in transport media, and laboratories only grow about 5% of the anaerobes present[21]. Swabbing does have the advantage of providing resistance and sensitivity information, but does not account for bacteria within biofilm. Clinical cultures really only provide information on the few bacteria that can be propagated efficiently in a laboratory[22]. Another disadvantage of clinical culture is that pathogens which constitute a small population in the wound but are present in high numbers may be overlooked[21].

If the swab result indicates a heavy growth of four or more bacteria, this may be equated to 10^5 colony forming organisms per gram of tissue. A result of 10^6 or greater indicates there are enough bacteria to cause deep tissue infection. Antimicrobial sensitivities will assist in determining the correct oral or parenteral antimicrobial treatment and highlight resistant organisms such as Methicillin-resistant *S. aureus*[14]. However, as many isolates are not identified to species level and numbers are not evaluated, the information given to the health care practitioner is usually insufficient to diagnose wound infection without relevant clinical signs and symptoms[11].

The number of organisms in a wound is now thought to be less important than the amount of different organisms in a wound. More than four different organisms are associated

with non-healing, with anaerobes having as much impact as aerobes[6].

Treating infection

> *Appropriate management of wound infection involves treating the right bacteria with the right agent/s, delivered in the right manner for the right length of time*[27].

Wound infections associated with systemic illness, deep tissue invasion or cellulitis require empirical systemic antibiotics. However, locally infected wounds will usually respond well to a topical antimicrobial, which, therefore, avoids the potential side effects of systemic antibiotics. Dressings containing cadexomer iodine and silver used at the correct time and in the correct concentration may help direct the wound bed in the desired direction, preventing infection and reducing the need for systemic antibiotics[28]. Topical antibiotics are not recommended due to inadequate penetration, development of antibiotic resistance, hypersensitivity reactions and local irritant effects creating further delays in healing[25].

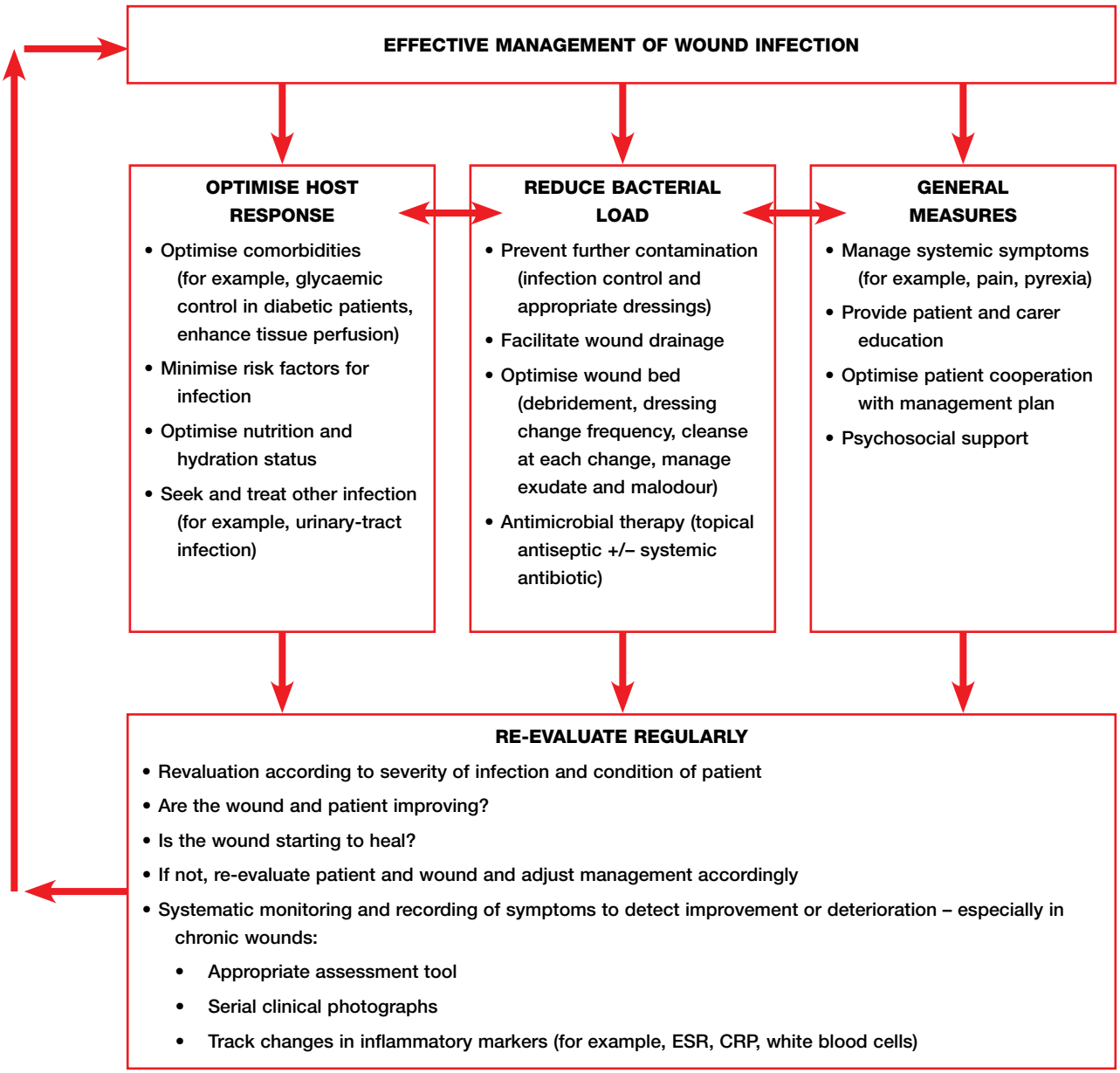
Effective management of infection includes: regular debridement of devitalised tissue (unless contraindicated); enhancing the patients' immune system; controlling co-morbidities and other factors that may delay healing; along with patient education and involvement[27] (Figure 1). Debridement physically reduces the bacteria and their secreted toxins, whilst also removing debris and devitalised tissue, hence reducing the nutrient source for remaining bacteria[2].

Diabetic foot ulcers

At least 50% of diabetic patients with a limb-threatening infection do not show systemic signs or symptoms, possibly due to reduced host inflammatory responses[11]. Therefore infection in diabetic foot ulcers (DFU) often can not be identified using usual clinical assessment and diagnosing infection before commencing treatment may not offer any benefit over empirical therapy[29].

The Wound Healing Society *Guidelines for the treatment of diabetic ulcers*[30] state that DFUs with "suspected" infection, or those not healing in a two-week time period, should be cultured to determine the microbial load. The guideline advises treating those ulcers with a microbial load greater than 10^6 organisms per gram of tissue or any beta haemolytic streptococci. This recommendation bases infection on wound

Figure 1. Management of wound infection (adapted from: Wound infection in clinical practice. An international consensus, 2008)



cultures, not clinical signs of infection[31] and highlights the complexity of assessing DFU infection, when normal clinical signs are often missing[32].

Biofilm

The harmful effect of microbial infection and the need to control bioburden are commonly accepted as important aspects of wound management. Until recently, it was believed that the properties of bacteria causing chronic infections were similar to those of free-floating, planktonic bacteria. However, research now indicates that many chronic infections result from bacteria living within a biofilm[33].

Individual organisms within a wound may not meet the requirements to cause infection, so they amalgamate to do this. They interact via quorum sensing (QS) which are communication systems that allow them to coordinate their activities and increase their capacity to cause disease[34].

Bacteria contain specific cell surface receptors which sense an appropriate environment to latch onto. Once attached to a surface, they surround themselves in a protective coating and form a biofilm. They then alter up to 50% of their bacterial proteins, making them quite different from their free-floating form[35], and this protects them from antibacterial agents and the immune system[16]. *Pseudomonas aeruginosa* expresses

73 extra genes when in a biofilm as opposed to when in a planktonic state[36]. Bacteria within biofilm need to be considered as being multi-celled, tissue-like structures rather than free-floating, independent pathogens[37].

It is thought that 60% of chronic wounds contain biofilm compared to 6% of acute wounds[13]. This may be due to the dynamic environment in an acute wound and the interaction between host proteins and bacterial contamination[35].

Wounds containing biofilm may have a bacterial count equal to or above the level for infection but not show usual clinical signs such as erythema, induration or pain[35]. Biofilm cannot be seen by the naked eye, but some clinical signs may include prolonged infection of over 30 days, poor response to antibiotics or resistance to antimicrobial dressings. The presence of slough, which is plasma that has been processed by bacteria, is also a common clinical sign, as is the wound that "waxes and wanes"[21].

Culture techniques cannot confirm whether bacteria have formed a biofilm and there is no routine way of detecting it[16]. Due to the complex casing protecting the microbes, laboratory specimens need to be broken down to break the carbohydrate bonds and release the organisms. Various methods have been attempted, including visualisation and molecular techniques, but these are prone to error and a standard method has yet to be developed[35].

Biofilm management is considered important, as they are associated with 80% of all known infections[23]. Mechanical debridement is an essential part of biofilm management. It aims to remove all necrotic tissue and biofilm and leave viable tissue untouched[35]. This also revitalises the host immune defences[38]. Other than sharp surgical debridement, there are various commercial products available including pulsed electric and radio frequency stimulation, pressure irrigation and ultrasound, but the latter two require further trials before being proven effective[35].

Topical antiseptics may be useful in preventing the re-formation of biofilm after debridement, but their effectiveness varies, and many are unable to penetrate the biofilm. Ionic silver is required in 10 to 100 times the strength used against planktonic bacteria. Some studies have shown that cadexomer iodine helps with biofilm suppression. Chlorhexidine and polyhexanide have been shown to disrupt microbial membranes but it is unclear as to their effect on biofilm. Leptospermum honey has been shown to be effective against up to 60 bacterial species, including in the biofilm form. Systemic antibiotics have as low as 25% to 30% efficacy in wound biofilm without evidence of systemic infection[38].

If 60% of chronic wounds contain biofilm, then more technical measures than wound swabs are required to accurately identify pathogenic wound biofilm. Procedures such as biopsy, light microscopy, scanning electron microscopy, epifluorescence microscopy techniques and molecular analysis have been suggested[36]. Damage to the QS systems may keep cells in the planktonic state. Therefore research is concentrating on QS inhibitors for potential new treatments, and a number of biofilm inhibitors are currently being investigated[39].

Questions to consider prior to diagnosing infection in chronic wounds

1. Clinical assessment – is the wound heavily colonised or infected?

2. NERDS or STONES?
3. Topical antimicrobials or systemic antibiotics?
4. Is a laboratory test appropriate? If so, which?
5. Can the underlying causes of chronicity be improved?

Conclusion

Wound infection is costly, both to the patient and health services. Clinicians should consider, assess for, and use preventative strategies to reduce the wound bio-burden. Laboratory tests are often necessary, but should not become a routine part of chronic wound care, as they cannot replace clinical assessment of the patient. However, clinical assessment is a judgement and open to error, as are the current wound sampling techniques. There is still much research needed before we can come up with a simple, fail-safe method of detecting early infection in all chronic wounds, but especially in people with diabetes.

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