REVIEW ARTICLE
TITLE: ASSESSMENT OF INFECTIONS IN SOFT TISSUE INJURIES FOLLOWING PENETRATING TRAUMA; A CLINICOLABORATORY DILEMMA.

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Introduction

Infection associated with penetrating trauma is generally a sequelea of accidents or violence.[1] In the event of infection, a wound fails to heal, the patient suffers increased trauma, treatment costs rise, and general wound management practices become more resource demanding.[2] In the face of increasing violence related injuries in our society and the consequent effects of infection on patient care, it is worthwhile to carry out this review on how soft tissue injuries should be assessed for infection following penetrating trauma.

The potential sources of wound infection in penetrating trauma before presentation in the hospital are: (1) the penetrating article introduced at the time of wounding, especially high velocity projectiles, which result in cavitation, (2) the environment where the trauma occurred and (3) the surrounding skin and endogenous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosae). The likely bacterial organisms that can pose an infection risk in traumatic injuries at the time of injury include:[2] *Clostridium tetani (C. tetani), Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus aureus (S. aureus), Staphylococcus epidermidis (S. epidermidis)*, *Propionibacteria,* Micrococci, Enterobacteriaceae and Skin diphtheroids. In hospitals, the factors that predispose to infection are multiple. The loss of the protective skin barrier such as in burns or avulsion injury accompanying the penetrating trauma and the presence of micro-organisms peculiar to the hospital environment especially multi-drug resistant organisms e.g. methicillin resistant *S. aureus,* vancomycin resistant *Enterococcus,* extended spectrum beta-lactamse (ESBL) producing enterobacteriaceae and carbapenemase producing enterobacteriaceae are known hospital factors that predispose to skin and soft tissue infections. In addition, foreign bodies such as implants and fixation equipment may be the source of infection in the hospital.

Penetrating Trauma

Penetrating traumas differ from blunt traumas, in that the skin is penetrated by the traumatic injury such as sharp objects and missiles. A critical aspect of penetrating trauma is the formula of kinetic energy, energy (E) = $\frac{1}{2}MV^2$, where M is mass and V velocity. Thus velocity of the penetrating object is a more important component of the injury than object size.[3] Injuries can be divided into very low energy such as knife or stab wounds, low energy such as handguns, and high energy such as military rifles. A review of the infection epidemiology in penetrating trauma shows very wide differences based on velocity of penetration. A large study of almost 40,000 knife wounds from Canadian emergency rooms failed to describe infection rates.[4] A recent review of 70 arrow injuries reported a 1.4% infection rate.[5] Stab wounds to the spine 'rarely become infected'.[6, 7] However, knife wounds of the abdomen are perhaps the exception; a recent paper where knife wounds comprised 19% of abdominal injuries described a 50% infection rate.[8] Blunt objects with irregular tips such as screwdrivers, which can introduce foreign material or in situations

where the penetrating object is contaminated also carry a huge potential for soft tissue infections.[1,9]

Although, in low energy injuries it is sensible to assume less infection potentials as they do not cause cavitation, therefore, allowing for all wound surfaces to re-approximate, creating a greater surface area in contact with the immune system, and preventing pathogen and foreign debris spread into surrounding tissue.[9]

Other sources of penetrating trauma include those from bullets, and those from fragmentation weapons such as grenades, antipersonnel mines or, as in recent asymmetrical warfare conflicts as seen in many parts of Northern Nigeria, the 'improvised explosive device' (IED).[10] IEDs are homemade using a variety of materials intended to penetrate, blast and burn with unique and unpredictable injury patterns. This differentiation is important as the resulting wound from each weapon is unique. Bullets have an available kinetic energy of 15003000 joules (J) for military rifles, 300500 J for handguns and 10150 J for some fragmentation devices.[11] This energy lacerates, contuses and displaces body tissues. In low energy wounds, injury is confined to the track of the projectile where the tissue is crushed by the bullet or fragment. High energy wounds produce radial injury around the track of the projectile with a temporary cavity where contaminants can be widely dispersed.[11] Furthermore, fragmentation devices create multiple wounds that are irregularly shaped and heavily contaminated with foreign bodies such as soil and clothing, with subsequent infection highly likely. Most bullets are pointed and initially transfer little material at entry. However, if cavitation occurs, bullet material may become widely dispersed, increasing infection risk.[11]

Bullets, despite being the product of an explosion that creates heat, were shown to be nonsterile in the 1960s.[12] Contamination notwithstanding, over 50% of bullet fragments are left unextracted without developing secondary infection, probably because metallic foreign bodies do not readily serve as a nidus of infection and have a low inflammatory potential.[13] Nonmetallic foreign bodies appear to be a greater cause of infection.[13] War wounds have the highest infection potential, owing to their high energy projectiles, a contaminated wounding environment and delay to definitive surgery compared with low energy civilian injuries.[14] A study of 17,726 combat wound casualties during the Vietnam war showed an infection incidence of 3.9% in the first 2 weeks after injury.[15] In reality, the incidence was probably higher because data was collected at an in-theater hospital; many patients lost to follow-up after 15 days might have gone on to suffer late infection.[1]

Although primarily young and without comorbidities, the penetrating trauma patient is often a diagnostic conundrum, and nowhere is this more apparent than in war situations.[1] This is due to the massive hemorrhage, tissue ischemia with impaired leukocyte and antimicrobial delivery to the affected tissues.[16] Furthermore, multiple transfusions are required, in effect causing a complete exchange transfusion, removing the victims' circulating immunity.[17] Recent studies indicate that severe blast injury (so prevalent in the current conflicts in several countries in Africa and Europe) overwhelms the immune system, thus sustained hyperinflammation ensues, which the host cannot physiologically regulate.[18] This dysregulation can lead to systemic inflammatory response syndrome (fever, hypotension and leukocytosis, among others) that may mimic infection, making diagnosis difficult and leading to inappropriate antibiotic administration.[19]

Conversely, these factors can also increase infection susceptibility at the penetrating injury site requiring antibiotic treatment.[20] Thus, the decision of when to administer antibiotics and what agent to select is an important and challenging one in penetrating trauma injury.

However, some pertinent issues complicate the assessment of a wound for infection. Health care practitioners often consider a microbiological report to provide definitive information on whether a wound is infected or not. An assumption that is incorrect as the wound could have been just contaminated or colonized by the microorganism as at the time of sampling. Therefore the provision of an antibiogram for any isolate can often be misleading and prompt unnecessary treatment. Moreover, infection in the immunocompromised hosts usually pose major diagnostic challenges for the following 3 reasons: (1) infections are caused by diverse organisms, including organisms not ordinarily considered to be pathogens in otherwise healthy hosts; (2) infection of the soft tissues may occur as part of a broader systemic infection; and (3) the degree and type of immune deficiency attenuate the clinical findings. The importance of establishing a diagnosis and performing susceptibility testing is crucial, because many infections are hospital acquired, and mounting resistance among both

gram positive and gram-negative bacteria makes dogmatic empirical treatment regimens difficult, if not dangerous. In addition, fungal infections may present with cutaneous findings in penetrating traumatic injuries in the presence of immuno suppression.

The assessment of a wound for infection is therefore best carried out by a combination of clinical and laboratory criteria.

Clinical Assessment Of Wound For Infection

Microbe-wound interaction can lead to three clearly defined outcomes[21]: (1) Contamination, (2) Colonization and (3) Infection.

Contamination

All wounds may acquire micro-organisms. If suitable nutritive and physical conditions are not available for each microbial species, or they are not able to successfully evade host defences, they will not multiply or persist; their presence is therefore only transient and wound healing is not delayed.[21]

Colonization

In colonization, microbial species successfully grow and divide, but do not cause damage to the host or initiate wound infection.

In this case, there is microbial growth, multiplication and invasion into host tissue leading to cellular injury and overt host immunological reactions. Wound healing is interrupted.

Wound infection is therefore the end result of a complex interaction between the host, organism, wound environment and therapeutic interventions, which is further complicated by bacterial cooperation and virulence. Recognition of subtle clinical changes in the inflammatory response will be necessary if the early signs of infection are to be identified

From the above it can be seen that not all isolates from a wound specimen are worthy of serious consideration. Therefore only wounds that show signs of infection should be sampled for microscopy, culture and sensitivity and the specimens should be appropriately collected.

Traditionally the presence of any of the following was used in determining wound infection: (1) Abscess (2) Cellulitis and (3) Discharge. However, in 1994, Cutting and Harding[22] published the following additional criteria: (4) delayed healing, (5) discolouration, (6) friable granulation tissue, (7) unexpected pain/tenderness, (8) pocketing at base of wound, (9) abnormal smell and (10) wound breakdown.

A delphi group was given the task of using the 10 features above in generating clinical criteria for infection in each one of the following six wound types: acute wounds (primary and secondary); arterial ulcers; burns (partial and full-thickness); diabetic foot ulcers; pressure ulcers and venous leg ulcers. Soft tissue injuries following penetrating trauma in this classification are secondary acute wounds. The features were graded as follows: 4-5 (**important**), 6-7 (**very important**), 8-9 (**diagnostic**). The structure of these bandings was driven by data.[23]

Score of 8 or 9

- Cellulitis
- Pus/abscess

Score of 6 or 7

- Delayed healing
- Erythema ± induration
- Haemopurulent exudate
- Increase in exudate volume
- Malodour
- Pocketing
- Seropurulent exudate
- Wound breakdown/enlargement

Score of 4 or 5

- Discolouration
- Friable granulation tissue that bleeds easily
- Increase in local skin temperature
- Oedema
- Unexpected pain/tenderness

The above criteria are used by the European Wound Management Association (EWMA) in the clinical assessment of wound infection. In addition to the above, the following features in a wound are indicative of anaerobic infection: foul odour of lesion or discharge, gas in tissues or discharges, tissue necrosis, gangrene, abscess formation, septic thrombophlebitis and black discoloration of blood-containing exudates.[23]

However, there are several intrinsic limitations to diagnosing a wound infection and establishing a treatment paradigm via clinical signs and symptoms alone. Of particular concern is the constantly evolving number of microorganisms with antibiotic resistance. The use of this method alone does not inform the wound care clinician of the most appropriate chemotherapeutic approach to treatment. Use of clinical signs and symptoms alone

leaves the provider to select a therapeutic agent based on little specific information about the particular pathogen(s). As a result, broad-spectrum chemotherapeutic agents may be initiated that only serve to facilitate the development of antibiotic resistance.[24] Furthermore, background immunosuppression in the patient may mask signs of wound infection. Skin lesions, no matter how small or innocuous in appearance, should be carefully evaluated, and the clinician must remember that their gross appearance is frequently altered by the decreased inflammatory response. Thus, the initial clinical impressions must be supplemented with a systematic approach for diagnosis and treatment.[25] After considering the important patient-specific factors concerning the patient's immune compromised status (e.g., neutropenia or neutrophil defects, cellular immune defect, and iatrogenic procedures), the gross morphologic characteristics of the skin lesion(s) should be characterized, the extent of the infection determined (e.g., localized vs. disseminated), and appropriate diagnostic tests undertaken to identify the infecting pathogen.[26]

Microbiological Assessment Of Wound For Infection

Principle of microbiology assessment

The principle governing the microbiology handling of specimens for analysis for wound infection is that micro-organisms resident on the skin and mucous membranes of humans as well as in the environment, can cause infection if they enter normally sterile tissue through breaks in the skin or normally intact mucous membranes. Because virulence mechanisms are not always necessary for each organism to cause infection in penetrating trauma, virtually any species can be involved. Interpretation of culture results should be based on gram stain criteria and extensive laboratory testing should be done only after consultation with the clinician.[27]

The Gram stain helps to determine the extent of workup required by culture. If abundant epithelial cells are seen, surface contamination is likely, and the isolates on culture should be minimally processed. The presence of numerous polymorphonuclear leucocytes or other phagocytic cells indicates an infectious process. On the other hand, if clinically important microorganisms are recognized or suspected on Gram stain e.g. *Clostridium*-like gram-positive bacilli, these should be immediately reported to the requesting physician even in the absence of phagocytic cells.

The Ideal Specimen

1) Wound biopsy/tissue: This is the ideal specimen. The skin surface and surgical area are disinfected before collection, and the specimen is obtained by an invasive technique (Please describe the technique). The disinfectant should be mild and not allowed to sip into deeper structures (i.e applied only on the wound surface and cleaned off immediately). Most normal microbiota is removed by this technique. Before a wound biopsy is taken, it is necessary for the wound care practitioner to find out if the microbiology laboratory can handle tissue specimens.[27,28]

2) Aspirated material from abscess, deep wounds, pus and exudates: This is the second best specimen. Uninvolved skin or mucous membrane should be thoroughly disinfected with alcohol followed by betadine before aspiration.[27,28]

3) Swabs: This is the least desirable because they hold the least volume and are most subject to contamination. Before a swab is applied, the surface should be cleaned with sterile saline using sterile cotton swabs and the skin surrounding the infected site should be disinfected as thoroughly as for an aspiration. Two swabs should be taken; one for microscopy and the other for culture.[27,28]

Blood for blood culture will be indicated if 2 or more of the following conditions develop in the patient;[29]

1. Temperature $>38^0$C or $<36^0$C
2. Heart rate >90 beats/min
3. Respiratory rate >20 breaths/min or $PaCO_2$ <32 mmHg
4. White blood cell count >12,000/≤ μL or <4000μL, or >10% band forms on differential count

Specimen Transport[27]

Tissue can be placed into a sterile container or Petri dish with a sterile moistened gauze pad. Formalin should not be used as this would affect the viability of the infecting pathogen. A transport medium that prevents overgrowth of rapidly growing microbes e.g. modified Stuart's or Amies, is recommended for swabs.

Specimen Processing[27]

a) ***TISSUE:*** (1) A small piece of intact tissue is used for an impression smear. (2) The remaining tissue is homogenized in broth. (3) The homogenate is used for staining procedures and culture. (4) Routine cultures are carried out on Blood Agar (BA), Chocolate agar (CHOC) and MacConkey agar (MAC) or Eosin Methylene Blue agar (EMB), plus

media for any other specific organism(s) sought.

b) ***SWABS:*** (1) Microscopy is carried out with one swab. (2) The second swab vortexed in broth and the extract used for culture. (3) Culture is carried out on BA, CHOC & MAC or EMB, plus media for any other specific organism(s) sought.

C) ***ASPIRATES:*** (1) Macroscopy. (2) Microscopy. (3) Culture is carried out on BA, CHOC and MAC or EMB, plus media for any other specific organism(s) sought.

Incubation Conditions[27]

BA and CHOC should be incubated in an atmosphere of 5-10% CO_2, while MAC can be incubated in aerobically at 35-37^{0}C for a minimum of 48 hours before discarding.

Wound infection by anaerobes

When an anaerobic wound infection is suspected, the microbiology laboratory should first be contacted to find out if the laboratory has the necessary set-up to isolate anaerobes. An anaerobic chamber is required in order to conclusively isolate an anaerobe in the microbiology laboratory. Thioglycolate broth or Robertson's cooked meat media can be used as a transport media for samples suspected for anaerobic infections.

Conclusion

I) Only wounds that are likely to benefit from a microbiological investigation should be sampled i.e., those with clinical signs of infection or those that are failing to heal.

2) A continuous dialogue between the microbiology department and the wound care practitioner is essential to ensure that:

a) The microbiologist has a thorough understanding of the clinical presentation of the wound.

b) The microbiologist has an understanding of the method of wound sampling.

c) The microbiologist is aware of the requirements of the practitioner and the urgency of the results; and

D) The practitioner understands the rationale for advice given by the microbiologist e.g. an antibiogram for *S. aureus* isolated from a mixed culture may not be provided if clinical signs of infection are not evident and if no inflammatory cells are seen in the Gram stain.

N B. The authors of this article would appreciate comments from readers.

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