The pathogenesis of tibial non-union

N Ferreira[1] BSc, MBChB, FC Orth(SA), MMed(Orth), PhD
LC Marais[1] MBChB, FCS Orth(SA), MMed(Ortho), PhD
C Aldous[2] BSc, BSc(Hons), MSc, PhD
[1] Tumour Sepsis and Reconstruction Unit, Department of Orthopaedic Surgery, Grey's Hospital, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal
[2] Medical Research Scientist, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal

Corresponding author:
Dr Nando Ferreira
Department of Orthopaedic Surgery
Grey's Hospital
3201 Pietermaritzburg
KwaZulu-Natal
South Africa
Tel: +27 33 897 3000
Email: Nando.Ferreira@kznhealth.gov.za

Abstract

Bone healing is a unique and complex reparative process that results in fractures healing without scar tissue formation. Multiple factors have been implicated in altering this process. This paper reviews the factors that influence the process of bone healing and predispose to non-union development. Cognisance of these factors will assist orthopaedic surgeons in identifying fractures at risk of altered healing and guide the development of comprehensive management strategies for established non-unions.

Key words: tibia, non-union, pathogenesis, fracture, healing

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Introduction

The human body has evolved the ability to spontaneously heal skeletal injuries through secondary bone healing and callus formation. This is evident from healed fractures observed in *Homo neaderthalensis* and *Homo erectus* fossils.[1,2] This healing process is unique in nature as most tissues heal with scar tissue formation, while skeletal tissue repairs with bone that is histologically indistinguishable from the original bone.

Manipulating this natural healing process in order to ensure proper alignment, maintenance of limb length and faster return to function, has been the goal of physicians throughout the ages. The Edwin Smith Papyrus from ancient Egypt is the oldest existing medical text and describes in detail the splinting of extremity fractures to preserve function.[3]

Non-union occurs when this natural healing process is hampered or disrupted and is one of the most dreaded complications of fracture management. Non-union following tibial shaft fractures represents the most common long-bone non-unions that require treatment.[4] Quoted incidences range from 4% to 48% and an established non-union signals a significant impact on a patient's function and quality of life.[4-9]

Multiple factors have been implicated in the pathogenesis of long-bone non-unions.[7,10,11] Recognising these factors will help refine strategies aimed at prevention of non-union and may guide the management of established non-unions. In this review we explore the factors that influence normal bone healing and predispose to non-union development after a tibial shaft fracture.

Normal bone healing

Bone healing is a complex cascade of events that results in the repair of fractures without the formation of scar tissue and can be classified into two histological types, namely primary and secondary bone healing.[12,13]

Primary bone healing ('soudure autogene') involves direct cortical remodelling through the formation of cutting cones that cross the fracture gap.[13] This type of bone healing occurs when there is a combination of anatomical reduction, stable fixation and compression of the fracture site and is only seen with open reduction and rigid internal fixation.

Secondary bone healing represents the most common type of fracture healing and occurs when there is some motion at the fracture site, which induces callus formation. During this healing process both endochondral and intramembranous ossification occur in an ordered sequence divided into three phases.[13]

The first phase starts with a haematoma that forms after the injury. This initiates an inflammatory response with the release of cytokines, including platelet derived growth factor (PDGF), TNF-α and interleukins from macrophages, neutrophils and platelets. These cytokines are responsible for the recruitment of fibroblasts and pluripotent mesenchymal cells that migrate to the fracture site. Granulation tissue forms around the fracture ends, and osteoblasts and fibroblasts proliferate. This is followed by the reparative phase when primary callus is formed. The mechanical environment drives differentiation of either osteoblastic or chondroblastic cell lines. Endochondral ossification mineralises a chondroid matrix while woven bone is generated through mineralisation of an osteoid matrix. The final stage involves remodelling the healed fracture site. This process is governed by Wolff's Law in response to mechanical stresses on the bone.

Mechano-biology

The mechanical environment plays a major role in fracture healing and can be described in terms of inter-fragmentary motion and strain.[14,15] While a small amount of relative deformation (strain < 2%) induces callus formation, high strain (> 10%) will lead to bone resorption and eventual non-union.[14] The amount of mobility allowed depends less on the displacement of the fragments alone than on the relation of the width of the fracture gap (L) and displacement (δL); δL/L.[15]

Mechanical stimulation also has a direct effect on the physiology of fracture healing. Ilizarov stated that functional load determines the structure, shape and volume of any limb. This is due to an increase in local blood flow during functional use that aids in tissue growth.[5] Mechanical stimulation also directly influences bone biology on a cellular level by stimulating the proliferation and differentiation of osteoblasts.[5,16] Mechanical force application patterns, as well as loading magnitude and frequency, also affect bone healing on a biochemical level.[16] The rates of synthesis and degradation of extracellular matrix components are affected by force application patterns. Loading magnitude affects cell size through increasing amounts of intermediate filaments and glycogen particles while changes in loading frequency can alter mRNA synthesis of anabolic and catabolic genes.[16] Aggrecan gene expression is increased in response to mechanical stimulation and leads to an increased proteoglycan scaffold for type II collagen.[5]

Mechanical stimulation has further benefits in terms of union site remodelling according to Wolff's Law. This phenomenon was originally ascribed to piezo-electrical charges that are generated in response to mechanical stresses. Osteoblasts on the compressive side are stimulated by electronegative charges while osteoclasts are activated by electropositive charges on the tension side.[17,18] This explanation is likely an oversimplification of a complex mechanism that regulates bone remodelling.[19] Current understanding of bone mechanosensation involves strain-generated potentials to explain how bone is able to respond to mechanical stresses.

Injury factors

The tibia is the most commonly fractured long bone.[4] Its anatomical location exposes it to high energy trauma and its thin soft tissue envelope means that these injuries are frequently open fractures.[20] This, along with a tenuous blood supply and complex fracture patterns that are frequently seen after high energy injuries predispose tibial fractures to complications that affect fracture healing.[5,8,13,20]

In an observational study of 200 patients, Bhandari *et al.* identified open fractures and transverse fracture patterns as independent variables that predict re-operation following tibial shaft fractures.[21] In this study, re-operation was defined as any surgical procedure aimed specifically at achieving bony union. In a more recent study, Fong *et al.* identified open fractures, comminution, fracture with less than 25% cortical contact, oblique fracture pattern and segmental fractures to be associated with non-union development. After multivariable logistic regression analysis only cortical contact of less than 25% remained as a variable that was a strong predictor of non-union and re-operation.[8]

The thin soft tissue envelope of the tibia is frequently breached during high energy trauma leading to these injuries being the most common open fractures managed by orthopaedic surgeons.[20] Open fractures result in loss of the initial fracture haematoma, periosteal stripping and ischaemic bone and soft tissues.[11] These factors contribute to an increased risk of non-union development in open fractures. Gaebler *et al.* found that grade III open fractures were five times more likely to develop delayed union compared to closed grade I and grade II fractures.[22] In a review of 104 patients, Karladani *et al.* reported a relative risk of 8.2 (95% confidence interval) for developing non-union in open fractures.[23] Gaston *et al.* reviewed 100 patients with tibial shaft fractures. They also reported a higher risk of non-union after open fractures with a relative risk of 3.4 (95% confidence interval).[24]

Atrophic non-unions in particular appear to be related to the extent of the initial damage sustained.[11,25] Injuries that result in extensive soft tissue damage, severe fracture comminution and devitalisation of fracture fragments have an increased risk of atrophic non-union.[5,25-27] Gaston *et al.* found that comminuted fractures had a higher likelihood of altered healing. They reported that Winquist and Hansen type III and IV tibial shaft fractures had 31% and 38% chance of non-union respectively compared to type I and II fractures that had an 8% chance of non-union each.[24] These high energy injuries appear to disrupt the vascularity of the fracture ends and affect the early stages of fracture healing.[11,28,29] In a rabbit model for atrophic non-union, the vascularity of the fracture site during the early stages of fracture healing was implicated as the driving force for atrophic non-union development.

This study found that although the non-union site appeared well vascularised at eight and 16 weeks, no vessels were seen within the interfragmentary gap at one week following the injury.[30]

The specific injury characteristics and damage sustained at the time of injury cannot be modified by the surgeon. Early identification of high-risk injury patterns should however prompt the treating surgeon to employ management algorithms that increase the chances of obtaining union.

Fracture management

Surgical intervention may inadvertently increase the chances of fracture non-union; the choice of fixation and the way in which it is executed can contribute to the overall risk of non-union. Fractures fixed in distraction, unstable fixation and excessive soft tissue dissection all contribute to an increased risk of non-union development.[13,25]

For fractures to heal, the mechanical environment must be appropriate.[31] Obtaining the ideal inter-fragmentary strain is of vital importance. Bhandari *et al.* identified fixation with a fracture gap as an independent risk factor for requiring additional surgery to achieve union.[21] Fracture gaps may potentially cause non-unions along two pathways. Unstable fixation coupled with small fracture gaps result in a high strain environment that favours chondroid and fibrous differentiation over osteogenesis.[25] Exposing the initial soft callus to excessive motion may disrupt the reparative phase of fracture healing and may result in a hypertrophic non-union.[27,32] On the other hand, fractures that are rigidly fixed in distraction may result in such low inter-fragmentary strain that no callus formation is stimulated. These situations often result in atrophic non-unions and fixation failure.

The optimal mechanical environment is however not the only consideration when deciding on fixation method, as this should be offset against preserving the remaining biological potential to unite. Open reduction and internal fixation might further disrupt a tenuous blood supply, especially in tibial fractures with concomitant soft tissue injury. Excessive stripping of soft tissue and periosteum may exacerbate necrosis of bone ends and contribute to the loss of biological potential to heal, ultimately resulting in an atrophic non-union.[25,29] Following high energy tibial fractures it might therefore be prudent to follow management strategies that preserve the local biological environment.

Host factors

Not all patients have the same fracture-healing potential. Some individuals have great ability to heal fracture gaps that might proceed to non-union in another person. The factors that contribute to impaired fracture healing include age, gender and certain concomitant systemic illnesses.[7]

Age

Age has a major influence on the body's ability to heal injuries. Children have a thick periosteum and an osteogenic environment dedicated to skeletal growth. This results in large haematomas and rapid callus formation after paediatric injuries.[33] As skeletally mature individuals advance in age a significant impact on skeletal repair is observed.[34,35] As a result, the observed healing time of fractures in the paediatric population is about half that in adults. Although there is no correlation between gender and non-union of fractures, healing problems are common among males since they have a higher incidence of high energy fractures.[31]

Concomitant systemic disease

- *Anaemia:* Low haemoglobin affects aerobic metabolic processes and alters the body's ability to repair injuries following trauma. Two animal studies investigated the effect of anaemia on fracture healing. Rothman *et al.* reported that iron-deficient anaemic rats had poor mineralisation of fracture callus and a decreased rate of union.[36,37] Heppenstall *et al.* found that hypovolaemic, anaemic rabbits showed inhibition of fracture healing but after fluid resuscitation, normovolaemic anaemic rabbits had no adverse effects.[38] Varecka *et al.* conducted a retrospective review of 734 patients and concluded that patients with a haemoglobin level below 8 g/dL had an increased risk of non-union. This was particularly significant in tibial fractures. In their series, patients that were smokers combined with anaemia had a 100% risk of non-union.[39]
- *Malnutrition:* Dietary and metabolic requirements increase during fracture healing.[11,25,31] Brinker *et al.* found that 85% of patients who developed unexplained non-unions had an underlying, undiagnosed metabolic or endocrine abnormality. The most common of which were vitamin D deficiencies.[10] Dodds *et al.* showed that vitamin B6-deficient rats had significant delays in callus maturation.[40] Osteoblast function has further been shown to be dependent on vitamin C.[41] In order to optimise fracture healing, patients should undergo careful nutritional assessment and any identified deficiencies should be addressed.
- *Diabetes:* Several clinical and experimental studies have shown that diabetes impairs bone healing.[31,42] Multiple animal studies using either rats with streprozotocin-induced diabetes or BB Wistar type I diabetic rats have investigated the effects of diabetes on fracture healing. These rats all show decreased callus stiffness and tensile strength in the early stages of fracture healing.[43,44] Diabetic rats were also found to have decreased cell proliferation, decreased collagen content and increased rates of cartilage resorption at the fracture site compared to controls.[43,45-47] Follak *et al.* showed that tight glycaemic control can produce normal fracture healing.[48] A study by Gandhi *et al.* further indicated that insulin might even play a direct role in healing at the fracture site.[49]

- *Hypothyroidism:* Urabe *et al.* investigated femur fracture healing in hypothyroid rats. They observed impaired healing as a result of deficient endochondral ossification. When these rats were treated with L-thyroxine, the healing process was returned to normal.[50]

The message from all these studies is clear: when confronted with a non-union, physicians should screen patients for these potential co-morbidities and all reversible or modifiable risk factors should be optimised during the healing process.

Smoking

Study data have conclusively revealed that smoking is associated with longer healing times, increased non-union rates and more wound complications after long-bone fractures.[26,51-54] The impact of smoking appears to be particularly pronounced in open tibial fractures.[55,56]

Several mechanisms have been proposed to explain how smoking impairs fracture healing and include alterations on a vascular, cellular and intracellular level. Smoking causes vasoconstriction and local hypoxia that could predispose the patient to atrophic non-union development.[11,57,58] Nicotine in tobacco prevents cellular proliferation, alters macrophage and fibroblast maturation and is directly toxic to proliferating osteoblasts.[11,31,59] Nicotine further inhibits TNF-α expression, required for fracture healing, through the activation of the cholinergic anti-inflammatory pathway.[60] On an intracellular level, smoking inhibits alkaline phosphatase and collagen production.[25]

Cobb *et al.* performed a case control study with patients undergoing ankle arthrodesis. They reported a relative risk of 3.74 for non-union in active smokers. When they analysed the patients without any other known risk factor for non-union development, the risk for non-union in smokers was 16 times that of non-smokers.[61] Bhandari *et al.* reported overall union rates of tibial shaft fractures to be higher in non-smokers (94%) when compared to smokers (84%).[21] Adams *et al.* showed that smokers had increased healing times after tibial fractures (32 weeks vs 28 weeks), required more bone graft procedures (26% vs 18%) and had a higher rate of non-unions, flap breakdown and infection.[55] A recent meta-analysis by Schenker *et al.* confirmed that the mean healing time for tibia fractures was longer for smokers (32 weeks) than for non-smokers (25 weeks) and that smokers with tibia fractures or open fractures had increased rates of non-union.[62]

Cessation of smoking may not result in an immediate improvement. Castillo *et al.* investigated patients who sustained open tibia fractures and found that current smokers were 37% and previous smokers 32% less likely to achieve union than non-smokers.[63]

It is clear from the available evidence that smoking negatively impacts healing of tibia fractures. It further appears that previous smoking negatively impacts outcome but to a lesser extent than current smoking. The question that remains to be answered is the time needed for the negative effects of smoking to dissipate after cessation of smoking. It is however prudent for physicians to encourage patients with acute fractures, and patients undergoing treatment for established non-unions, to stop smoking.

NSAIDs

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used to manage post-traumatic or post-operative pain. They inhibit cyclooxygenase (COX) enzyme activity and decrease prostaglandin production, which may have a detrimental effect during the inflammatory phase of fracture healing. Conflicting evidence about their effect in clinical practice however remains.[64,65]

Multiple clinical trials have failed to provide a definitive answer to the effect of NSAIDs on fracture healing.[66] Burd *et al.*, Giannoudis *et al.* and Bhattacharyya *et al.* all reported significant risk for non-union of long bone fractures with the use of NSAIDs.[67-69] Davis *et al.* and Adolphson *et al.*, however, failed to show any correlation between the use of NSAIDs and abnormal fracture healing.[70,71] It is notable however that both these studies were conducted on patients who sustained Colles' fractures that generally are unlikely to develop non-unions. Studies investigating the effect of NSAIDs on spinal fusion also failed to provide conclusive answers, with some studies showing an inhibitory effect toward fusion while others contradict these findings.[72-76]

In vitro and animal studies has shown similar variations in outcome.[64,65] The diversity in study design may have contributed to the lack of consensus, but even studies with identical study parameters sometimes report contradictory findings.

Conclusive evidence against the use of NSAIDs in acute fracture care cannot be drawn from the available evidence. The lack of evidence is, however, not proof of the absence of a detrimental effect and these drugs should be used with caution in patients with high-risk for abnormal fracture healing.[64]

Other drugs

- *Antibiotics:* Animal and in vitro evidence indicate that antibiotic therapy may have adverse effects on fracture healing.[77-79] The quinolones, ciprofloxacin, levofloxacin and trovafloxacin have been shown to decrease cellular proliferation and DNA synthesis which result in diminished healing during the early stages of fracture repair.[77,78] The aminoglycosides gentamycin and tobramycin decrease proliferation of osteoblastic progenitors and are directly toxic to osteoblasts.[80,81] Experimental studies have shown that osteoblast proliferation might be inhibited by rifampicin at clinical doses.[82] There is however little evidence on the effect of antibiotic therapy on fracture healing in humans.[79]

- *Anticoagulants:* In vitro and in vivo evidence suggest that some anticoagulants may impair normal bone metabolism.[83-85] Several animal studies have demonstrated significant attenuation of fracture healing but no human trials are available for evaluation.[5,31,83,85] A literature review by Lindner *et al.* identified strong evidence that warfarin and heparin retard fracture healing, but low molecular weight heparins appear to have a less pronounced effect.[86]
- *Anticonvulsants:* There is a growing body of evidence on the adverse effects of anticonvulsants in bone metabolism. Phenytoin, phenobarbital, carbamazepine primidone and valproate have all been implicated in causing decreased bone mineral density and disorders of bone metabolism.[87-91] The extent to which these drugs affect fracture healing in humans remains to be evaluated.
- *Chemotherapy:* Chemotherapeutic agents significantly affect fracture healing. Their cytotoxic and anti-proliferative properties impact neovascularisation and callus formation resulting in higher non-union rates.[31,92] Cyclophosphamide causes diminished calcium and phosphate deposition in callus.[93] Doxorubicin, cyclophosphamide, adriamycin and methotrexate results in decreased bone formation and these effects might last up to three weeks after administration.[93]
- *Corticosteroids:* The effect of long-term corticosteroid use on bone metabolism and fracture healing is well documented.[31,94,95] The long-term use of corticosteroids leads to osteoblast and osteocyte apoptosis and inhibition of osteoblastogenesis.[5,13,92] Waters *et al.* studied the effects of long-term steroid use on fracture healing in a rabbit model. They found an 85% rate of non-union in the corticosteroid group compared with 18% in the control group.[94] In contrast, Hogevold *et al.* investigated short-term corticosteroids use on fracture healing in rats and found no statistically significant difference when compared to a control group.[96]

Alcohol

Chronic alcohol consumption leads to osteopaenia, increased risk of fracture from falls and delays in fracture healing.[97] Many of these problems have been attributed to nutritional deficiencies and biochemical derangements frequently observed in chronic alcohol abuse.

Recent research has, however, illustrated that excessive alcohol use may have a direct impact on bone healing. It appears that excessive doses of ethanol in the early healing period inhibit new bone formation and that the newly formed bone lacks mineralisation, causing decreased stability and leading to increased incidence of delayed union.[5,11,31,92]

Experimental evidence from ethanol exposed fracture healing in murine models indicates that ethanol impairs the biomechanical strength and decreases the volume of callus formation.[98-100] Chakkalakal *et al.* studied the effects of ethanol on a fracture model in rats. They found that rats that were fed ethanol as 35% of their total calorie intake had deficient bone repair that could not be attributed to nutritional deficiencies. They further found that removal of ethanol from the diet after the bone injury completely restored bone healing.[99]

A retrospective study by Askew *et al.* was consistent with these animal findings. The investigators compared the healing time of fractures in 12 alcoholics and 18 non-alcoholics and found delayed healing time in alcoholics of more than twice that of non-alcoholics.[101]

These studies indicate that alcohol might have a direct negative effect on fracture healing. It appears, however, that these effects could be negated by the early cessation of alcohol intake following an injury.

Infection

Sepsis is often cited as a cause of non-union development.[13] Infection and non-union does not, however, have a simple cause-and-effect relationship. Many factors that promote infection, like open wounds with extensive devascularisation, tissue necrosis and instability, are also implicated in non-union development.[11,25] Infection can however contribute to non-union development through bone death, creation of fracture gaps due to bone resorption, and instability because of implant loosening.[25]

Human Immunodeficiency Virus

HIV infection has recently been disputed as a risk factor for non-union development. Initial studies showed an increased risk for non-union in certain HIV-positive subgroups. Kamat and Govender evaluated the effect of HIV infection on union rates of closed ankle fractures that were managed non-operatively. They concluded that there was no difference in union rates of HIV-negative and WHO clinical stage I, II and III HIV-positive patients, while patients with WHO clinical stage IV HIV infection had increased non-union rates. (12.45% vs 1.5% and 1.25%)[102] Chandanwale *et al.* compared healing rates in 80 HAART naive HIV-positive patients with 80 HIV-negative controls. Closed fractures had similar healing rates in the two groups when treated conservatively or operatively. Open fractures in the HIV-positive group, on the other hand, showed a significantly increased risk of non-union. (50% vs 15%)[103] Aird *et al.* prospectively evaluated 133 patients (33 HIV-positive) with open fractures. They reported a non-union risk of 15% in HIV-positive patients compared to 4% in HIV-negative patients.[104]

More recent research has contradicted these earlier findings. Gardner *et al.* prospectively evaluated union in 96 HIV-positive patients. They reported that 4% of these fractures failed to unite and concluded that HIV infection did not increase the risk of non-union in surgically managed fractures. This cohort, however, included only five open fractures.[105]

The exact mechanisms by which HIV infection affects fracture union remain unclear although multiple pathways have been suggested. Molecular and biochemical hypotheses could explain a direct relationship between HIV and impaired fracture union. HIV infection is known to cause an altered cytokine environment that may impact bone healing. TNF-α is up regulated while IGF-1 levels are reduced and an inverse correlation between IGF-1 and IL-6 is observed when compared to HIV-negative individuals.[106] HIV may further affect fracture healing by its impact on general health through malnutrition, reduced body mass and opportunistic infections.

Considering the limited and controversial evidence regarding fracture healing in HIV-infected individuals, it might be well advised to take particular care to optimise bone healing in HIV-positive patients. A tailored fracture management strategy, improvement of nutritional status, avoidance of NSAIDs and cessation of smoking and alcohol consumption might assist in mitigating the potential negative effects of HIV infection on bone healing.

Genetics

Despite the lack of any apparent risk factors, some patients still proceed to non-union development.[107] This has led to the hypothesis of a genetic predisposition to altered fracture healing.[108]

Zeckey *et al.* identified a significant correlation between polymorphisms in the PDGF gene and non-union development after femoral and tibial shaft fractures.[109] Dimitriou *et al.* investigated the impact of genetic defects in the BMP signalling cascade on non-union development. The study identified two specific single nucleotide polymorphisms on the *NOGGIN* and *SMAD6* genes that were associated with an increased risk for atrophic non-union development.[110] Fajardo *et al*. examined RNA expression patterns of BMPs, their receptors and inhibitors in hypertrophic non-union tissue. They found substantially elevated concentrations of BMP-4 and certain BMP inhibitors (Drm/Gremlin, follistatin and Noggin) while levels of BMP-7 was lower than those seen in normal fracture healing.[111]

The extent to which these genetic components predispose to non-union formation, and their role and interaction with other risk factors, warrant further investigation.

Conclusion

Non-union development has a multifactorial pathogenesis that is not well understood. The weight that each variable contributes to non-union development remains unclear and a cumulative effect to the development of a non-union is probably involved. A greater understanding of the contributing factors to non-union development will assist orthopaedic surgeons in identifying fractures at risk of altered healing, and assist in the development of multidisciplinary management strategies for established non-unions.

Conflict of interest statement
The authors declare that they have no conflict of interests and that no financial support was received for this study.

Authors' contributions
All three authors made contributions toward the conception and design of the research, acquisition of data and drafting of the manuscript. The final manuscript was read and approved by all the authors.

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