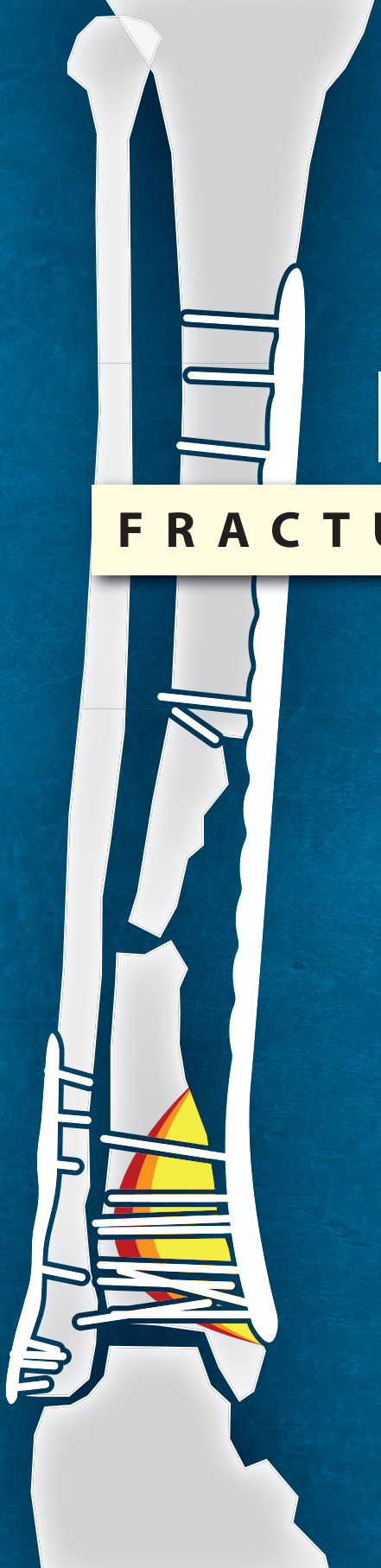

DIAGNOSING FRACTURE-RELATED INFECTIONS

Getting it right first time

GEERTJE GOVAERT

It is difficult to treat a disease that has not been properly diagnosed.

Geertje Govaert

Diagnosing fracture-related infections, getting it right first time.

Thesis, Utrecht University, the Netherlands, with summary in Dutch.
Proefschrift, Universiteit Utrecht, Nederland, met een samenvatting in het Nederlands.

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Diagnosing fracture-related infections
getting it right first time

Het diagnosticeren van fractuur-gerelateerde infecties
doe het in één keer goed
(met een samenvatting in het Nederlands)

P R O E F S C H R I F T

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op
donderdag 29 november 2018 des middags te 4.00 uur

door

Geertruida Anna Maria Govaert
geboren op 30 december 1972 te 's-Hertogenbosch

Promotor:	Prof. dr. L.P.H. Leenen
Copromotoren:	Dr. A.W.J.M. Glaudemans Dr. F.F.A. IJpma

Twenty years from now you will be more disappointed by the things that you didn't do than by the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails.

Explore. Dream. Discover.

H. Jackson Brown Jr. in: P.S. I Love You, 1991

Voor mijn ouders
en peetouders †

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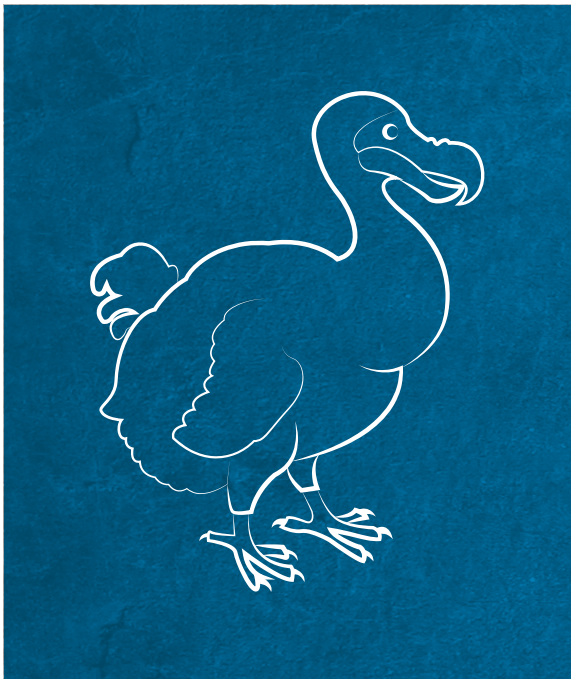

PART I

THE PROBLEM

CHAPTER 1

General introduction and outline of this thesis

BACKGROUND

Infection of the bone after an open fracture has existed as long as vertebrate life on Earth has prevailed (Figure 1) [1, 2]. In the first half of the nineteenth century the foundations were laid for the modern treatment of injuries of bones and joints. Early obstacles to the development of operative treatment included the pain associated with surgery and, significantly, concerns about infection and its potentially fatal consequences. After the introduction of anaesthesia (1846), antisepsis (1867), X-rays (1895) and, finally, penicillin (1928), operative stabilisation of fractures became a real option for the surgical pioneers of that time [3]. Thanks to these developments in medicine more fracture surgeries were being performed. This rise in surgical procedures was naturally accompanied by the corresponding burden of surgical complications. Postoperative infection has always been the most challenging of these complications.

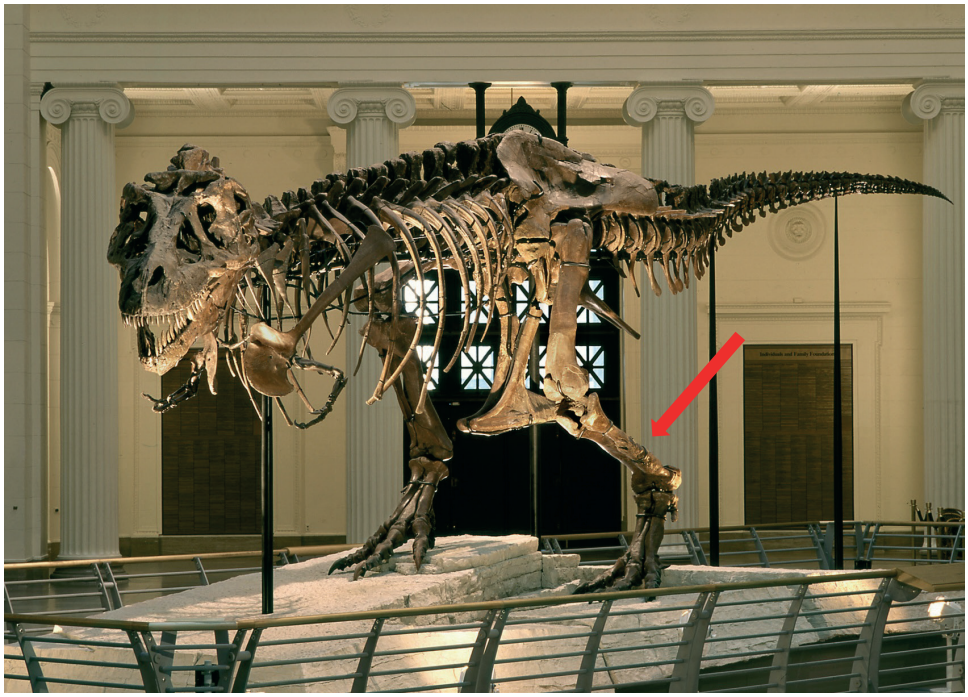

Figure 1. Sue. Sue is the nickname given to the largest, most extensive and best preserved *Tyrannosaurus rex* specimen ever found. It was discovered in 1990, by Sue Hendrickson, an explorer and fossil collector, and was named after her. A variety of abnormalities are present throughout the skeleton. These include healed fracture-related infections of the left fibula (red arrow) and proximal right humerus and multiple bilateral rib fractures.
SUE 1 (c) The Field Museum, Chicago, Illinois, USA (reproduced with permission)

Definition

Until very recently, many different definitions of infection following surgical fracture care were used. Terminology such as osteitis, osteomyelitis, posttraumatic osteomyelitis, implant-related infection, deep infection and many more were applied randomly without uniform agreement on an actual definition of this complication [4]. This is confirmed in a systematic review showing that only a minority (2%) of randomised controlled trials on fracture care use any kind of standardised definition of infection [5]. The same applies to the distinction between early and delayed, or acute and chronic infections. This commonly used time-related distinction is rather arbitrary and has never been validated towards outcome of treatment of the disease. Since the turn of the millennium there has been growing awareness of the need for a widely supported consensus on the definition of infection after fracture care. This allows medical practitioners to compare the results of future research and the subsequent development of international protocols and guidelines. For this reason, in 2017 a consensus definition including diagnostic criteria was proposed by a group of experts representing the Association for the Study of Internal Fixation (*Arbeitsgemeinschaft für Osteosynthesefragen*, AO), the European Bone and Joint Infection Society (EBJIS), and prominent orthopaedic trauma hospitals and academic centres. This definition was published in 2018 [6]. From that publication onwards, fracture-related infections (FRI) encompass the complete spectrum of infections (e.g. acute or chronic, superficial or deep, with or without bone involvement, with or without implants in situ) following surgical fixation of a closed or open fracture. Two levels of certainty were defined around the diagnostic features of FRI. Criteria could be confirmatory (infection definitely present) or suggestive (infection possibly present). Confirmatory criteria consist of 1) a fistula, sinus or wound breakdown (with communication to the bone or implant), 2) purulent drainage from the wound or presence of pus during surgery, 3) phenotypically indistinguishable pathogens identified by culture from at least two separate deep tissue/implant (including sonication-fluid) specimens taken during an operative intervention, and/or 4) presence of microorganisms in deep tissue taken during an operative intervention as confirmed by histopathological examination. Suggestive criteria for FRI comprise 1) clinical signs (e.g. pain, local redness, local swelling, fever), 2) radiological signs (e.g. bone lysis, implant loosening, sequestration, non-union, unexpected periosteal bone formation), 3) a pathognomonic organism identified by culture from a single deepimplant/tissue specimen, 4) elevated serum inflammatory markers, 5) persistent, increasing or new-onset wound drainage beyond the first days postoperatively, and/or 6) new onset of joint effusion in fracture patients.

Incidence and risk factors

On average, the incidence of FRI is 1-5% [7, 8] with outliers up to 45% in case of very severe tissue damage and/or contaminated wounds [9, 10]. The incidence of fractures in men is 11.67/1000/year and in women 10.65/1000/year [11]. The exact number of all fractures that need surgical stabilisation is unknown. As a rule of thumb it is reported that one-third of all patients with a fracture need hospitalisation and that 80% of these admitted patients need surgical fracture care [12, 13]. This implies that in the Netherlands (the Dutch population in 2018 was roughly 17.2 million) approximately 3000 patients are diagnosed with FRI every year, 0.5 patients per family practice and 20 patients per hospital [14, 15]. The number of *suspected* FRIs is unknown. Many patients are treated in an ambulatory setting with an empiric antibiotics course for suspected yet unconfirmed "superficial wound infections". In a majority of these patients management with antibiotics is likely unnecessary. Even in a study with a group of 192 highly selected patients undergoing white blood cell scintigraphy for suspected FRI, only one third subsequently confirmed infection [16].

Known risk factors for the development of FRI in tibial fractures include previous need for an external fixator (this implies either a severely injured patient or extensive soft-tissue damage), time to nailing, open fractures and subsequent Gustilo-Anderson grade [17]. In open fractures, reported risk factors for the development of an FRI are male gender, diabetes mellitus, smoking, lower extremity fracture, Gustilo-Anderson grade 3 open fracture, contaminated fracture and polytrauma [18].

Diagnosis

It is difficult to treat a disease that has not been properly diagnosed. One of the challenges in orthopaedic trauma care is that FRI can present itself in many different ways. Sometimes the clinical scenario is clear and the diagnosis can be made on clinical examination only. This is the case with confirmatory clinical criteria such as a fistula or pus drainage from the wound. It is also possible for the presence of an FRI to be more obscured and for suggestive signs such as redness, swelling or pain to be mimicking a non-infected condition (such as posttraumatic arthrosis or a non-infected delayed fracture union). Many authors have acknowledged that the most optimal diagnostic strategy for FRI remains unclear [19]. In fact, most recommendations for diagnostic workup of FRI are based on level-4 evidence, small series, expert opinions and local consensus meetings [7, 20-22]. This mirrors the situation for periprosthetic joint infections (PJI) seven years ago. In 2011, a workgroup convened by the Musculoskeletal Infection Society (MSIS) published a consensus definition for PJI in order to support clinicians' diagnostic confidence and to promote uniform gold standards for further

research [23]. This dedicated approach led to a multi-institutional research collaboration and publication of a recent update on diagnostic criteria for hip and knee PJI [24]. With the publication of the consensus definition for FRI hopefully the first step is being taken towards a similar development for this condition, which will subsequently lead to evidence-based guidelines in the near future.

OUTLINE OF THIS THESIS

The overall aim of this thesis is to improve the diagnostic process for FRI. The challenges in the diagnostic workup of an FRI can be ascribed to different aspects of this process. This thesis is therefore divided into six parts.

Part I outlines the problem. There is a lack of evidence for diagnosing FRI (Chapter 1). This results in a lack of consensus on diagnostic strategies for FRI and limited awareness towards uniform FRI protocols (Chapter 2).

Part II focuses on medical imaging. First, the concept of nuclear imaging of posttraumatic osteomyelitis (later renamed FRI) is explained (Chapter 3). In order to understand the current evidence on the diagnostic accuracy of imaging modalities for posttraumatic osteomyelitis/FRI, the available literature is systematically reviewed (Chapter 4). Finally, the diagnostic accuracy of the two most commonly used nuclear imaging modalities for FRI – white blood cell (WBC) scintigraphy and ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) – are investigated (Chapter 5 and Chapter 6).

Part III reports on the use of serum inflammatory markers. The diagnostic accuracy of serum inflammatory markers is investigated in a large patient cohort (Chapter 7); this is followed by a systematic review and meta-analysis of the literature (Chapter 8).

Part IV focuses on microbiology. Infection is all about pathogens, and those pathogens need to be accurately identified. The importance of applying a structured tissue sampling protocol is discussed (Chapter 9) and the accuracy of tissue and sonication fluid sampling is systematically reviewed (Chapter 10).

Part V deals with implementation of the gathered knowledge and looks into the future. The recently published Dutch guideline on FRI is summarised (Chapter 11) and an outline is presented of a trial protocol for a prospective study to investigate the accuracy of diagnostic imaging techniques for FRI (Chapter 12).

Part VI is the general discussion, providing an overview of current concepts regarding FRI diagnosis and ending with final thoughts and future perspectives (Chapter 13).

A summary of the research questions addressed in this thesis is presented in Table 1.

Table 1. Summary of research questions addressed in this thesis.

Chapter		
2	-	What are the currently preferred imaging strategies for diagnosing post-traumatic osteomyelitis (PTO) among orthopaedic and trauma surgeons, radiologists and nuclear medicine physicians?
	-	What is the preferred serum inflammatory marker for diagnosing PTO, and are there local hospital protocols to diagnose and manage PTO?
3	-	What are the three most commonly used nuclear medicine techniques about: three-phase bone scan (with SPECT-CT), white blood cell (WBC) scintigraphy with SPECT-CT, and ^{18}F-fluorodeoxyglucose (FDG)-PET/CT?
4	-	What is the current evidence on imaging techniques to diagnose PTO?
5	-	What is the accuracy of WBC scintigraphy for diagnosing fracture-related infections (FRI)?
	-	Does the duration of the time interval between surgery and WBC scintigraphy influences its diagnostic accuracy?
6	-	What is the diagnostic performance of ^{18}F-FDG-PET/CT for diagnosing FRI?
	-	What is the diagnostic performance of Standardized Uptake Values (SUVs) in ^{18}F-FDG-PET/CT for diagnosing FRI, and what are their associated cut-off values?
	-	Which variables are independent predictors of a false-positive or false-negative test result in patients with suspected FRI?
7	-	What is the diagnostic accuracy of the two commonly used serum inflammatory markers, CRP and leukocyte count, in patients presenting with suspected FRI?
8	-	What is the current evidence on the diagnostic value of CRP, leukocyte count and ESR in FRI?
9	-	What is the effect of a structured microbiology sampling protocol for FRI compared to ad-hoc culture sampling?
10	-	What is the current evidence of validation studies regarding sonication fluid cultures, molecular techniques and histopathology as diagnostic criteria for FRI?
11	-	What is the content of the new Dutch guideline on diagnosis and treatment of FRI?
12	-	What is the next step for future research in determining which imaging modality has the highest diagnostic accuracy for FRI (presentation of the 'Imaging of Fracture-related Infections (IFI)' trail protocol)?
13	-	What are the evidence-based current concepts for diagnosing FRI?

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CHAPTER 2

Diagnostic strategies for posttraumatic osteomyelitis; a survey amongst Dutch medical specialists demonstrates the need for a consensus protocol

G.A.M. Govaert, A.W.J.M. Glaudemans, J.J.W. Ploegmakers, R. Viddeleer, K.W. Wendt, I.H.F. Reininga

Eur J Trauma Emerg Surg. 2018 Jun;44(3):417-426

ABSTRACT

Introduction. Posttraumatic osteomyelitis (PTO) is a feared complication after surgical fracture care. Late diagnosis can result in interrupted and prolonged rehabilitation programmes, inability to work, medical dependency, unnecessary hospital admissions and high medical and non-medical costs. Primary aim of this study was to assess preferred diagnostic imaging strategies for diagnosing PTO amongst orthopaedic and trauma surgeons, radiologists and nuclear medicine physicians. Secondary aims were to determine the preferred serum inflammatory marker for diagnosing PTO and the existence of a hospital protocol to diagnose and manage PTO.

Material and Methods. This study utilised an online survey based on 4 clinical scenarios, varying from early to late onset of PTO. It was designed to assess individual practitioners' current preferred diagnostic strategy for diagnosing PTO. Eligible study participants were medical specialists and registrars in orthopaedic and trauma surgery, musculoskeletal (MSK) radiology and nuclear medicine.

Results. There were 346 responders: 155 trauma surgeons, 102 orthopaedic surgeons, 57 nuclear medicine physicians and 33 musculoskeletal (MSK) radiologists. Trauma surgeons favour FDG-PET to image PTO, while orthopaedic surgeons prefer white blood cell (WBC) scintigraphy. A similar difference was seen between radiologists and nuclear medicine physicians (MRI versus nuclear medicine imaging). C-reactive protein was regarded as the most useful serum inflammatory marker. Only one-third of all responders was aware of a hospital protocol for the treatment of osteomyelitis.

Conclusions. The availability of and awareness towards local protocols to diagnose and treat PTO is poor. The results of this study support the need for future randomised controlled trials on optimal diagnostic strategies for PTO.

INTRODUCTION

The reported incidence of 1–19% of deep infections after surgical fracture care is much higher than in procedures such as elective orthopaedic joint replacement (reported infection rate: 0.8–1.2%) [1-4]. This is not surprising, not only because of the typically acute setting in which trauma surgery takes place but also because of numerous other contributing causes. The nature of a fracture (anatomic location, open versus closed, high-energy versus low-energy impact), level of wound contamination in open fractures, systemic inflammatory response due to soft-tissue injury, possible accompanying vascular injury, timing and duration of surgery, and severity of concomitant injuries (which can require a hasty damage control procedure) are all factors that influence the risk of developing a deep fracture-related infection [1, 2, 5-11]. Diagnosing fracture -related osteomyelitis, also referred to as posttraumatic osteomyelitis (PTO), is challenging and requires in-depth knowledge of the problem as well as a high index of suspicion by the treating medical team [12, 13]. A surgical site infection (SSI) is usually easily recognisable by the four classical signs of infection: calor, dolor, rubor and tumor. This is rarely the case for a long-standing PTO which can present with a closed wound and no apparent acute signs of infection. Symptoms such as pain and disability to use the affected limb can mimic other differential diagnoses like non-infected non-union, posttraumatic arthrosis or simply symptomatic hardware.

Most recommendations for the best diagnostic workup of PTO are at best level-4 evidence, based on expert opinions and local consensus meetings [7, 14-17]. Serum inflammatory markers such as leukocyte count (LC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are widely used but their diagnostic value for PTO is poorly studied. The same can be concluded for medical imaging modalities. With recent developments in hybrid camera systems such as Single Photon Emission Computed Tomography combined with Computed Tomography (SPECT-CT) and Positron Emission Tomography combined with CT (PET-CT), there are now more advanced methods to image PTO [17]. These newer techniques achieve a higher diagnostic accuracy by combining pathophysiology with anatomy in a single imaging modality. Although they are already used on a large scale worldwide, these modern imaging modalities are not yet prospectively studied in large PTO patient populations and therefore have not yet been implemented in evidence-based guidelines. Most clinicians acknowledge the fact that every imaging technique has its advantages and disadvantages, and rely on local customised preferences and logistic availability. X-rays and CT are useful to assess the position of metal implants, fracture stability and bone healing. Osteomyelitis can sometimes be detected by periosteal reaction, cavities and a fuzzy appearance of the cortex, but the sensitivity and specificity are low [18] and radiologically detectable

changes appear much later than the onset of the infection. MRI is useful as it differentiates necrotic from viable tissues and assesses the extent of infection. It is sensitive for detecting osteomyelitis but its diagnostic accuracy decreases after recent surgery, when metal implants are present and differentiation between sterile inflammation and still-existing infection is difficult [19-21]. The same applies to three-phase bone scintigraphy: although it is useful when negative, it has a very low specificity in the acute/subacute setting as any recent alteration to the bone will result in a positive outcome [22]. White blood cell (WBC) scintigraphy has been extensively studied for peripheral osteomyelitis and is found to be reliable with high overall accuracy rates [23, 24]. All these studies, however, were conducted on heterogeneous patient groups, including joint prosthesis infection and diabetic feet, and none focused specifically on suspected PTO. The diagnostic value of PET/CT with ^{18}F-fluorodeoxyglucose (FDG) for osteomyelitis is still under investigation. This technique has the best properties: easy labelling procedure, available in many centres and short imaging time. Unfortunately, the problem with FDG is that it is aspecific: it accumulates in healing tissues, in inflammation and in infection, which has led to a huge variation in reported sensitivity and specificity values for osteomyelitis. Furthermore, no interpretation criteria presently exist as to when to declare a FDG-PET positive or negative for infection.

As a baseline for future research and for the development of a national protocol on posttraumatic osteomyelitis we conducted this inventory study. Our primary aim was to assess current preferred imaging strategies for diagnosing PTO amongst orthopaedic and trauma surgeons, radiologists and nuclear medicine physicians. Secondary aims were to determine the preferred serum inflammatory marker for diagnosing PTO and the existence of a local hospital protocol to diagnose and manage PTO.

MATERIALS AND METHODS

Participants and data collection

This study utilised an online sixteen-question survey (the diagnostic osteomyelitis survey) designed to assess individual professionals' current preferred strategy for diagnosing PTO. Eligible study participants were Dutch consultants and registrars in orthopaedic and trauma surgery, musculoskeletal (MSK) radiology and nuclear medicine. Requests for participation (followed by two reminders in case of no response) were sent via an email that described the outline of the study and its aim, with an invitation to complete a web-based survey. A total of 2,343 invitations were sent to members of the four medical professional associations: the Dutch Society for Trauma Surgery (NVT; 581 invitations), the Dutch Orthopaedic Society (NOV; 1,331 invitations), the Dutch Society of Nuclear

Medicine (NVNG; 161 invitations) and the musculoskeletal (MSK) section of the Dutch Radiology Society (NVvR; 270 invitations). In the Netherlands there are 133 hospitals, eight of which are University Medical Centres (UMC) and 28 large peripheral teaching hospitals (PTH) [25] . In this study the remaining 97 smaller hospitals were regarded as peripheral non-teaching hospitals (PNTH), as the gamut of medical specialist training possibilities at such hospitals is limited or absent.

We developed the web-based diagnostic osteomyelitis survey using the secure Share Point Server 2013 of University Medical Center Groningen (UMCG) in the Netherlands; the survey was presented to the respondents using an *https connection*. All data were de-identified and stored securely on the UMCG server; access was restricted to the research team. The local UMCG medical ethical committee judged the methods employed in this study and waived further need for approval (reference number METc2014.554).

The diagnostic osteomyelitis survey

The survey consisted of 16 questions and took approximately 10 minutes to complete. For some questions more than one answer option could be selected. Demographic data of the responders were collected, including profession and hospital-specific data.

To assess the current preferred imaging strategies of the responders as realistically as possible, four patient-based clinical cases were presented. Each case described a patient with a different stage of fracture-related osteomyelitis, representing a typical clinical scenario (Fig 1). These patients gave written consent for the anonymous use of their medical imaging. Each case was introduced with the relevant medical history of the patient combined with a clinical picture of the affected limb. Patient A had an acute surgical site infection (SSI) one week after open reduction and internal fixation (ORIF) of a distal humerus fracture. Patients B, C and D had a suspected (patient B) or obvious (patients C and D) late infection after surgical fracture care. The distinction between patients C and D was the presence of metal implants.

X-rays of the fracture site were only provided if the participants selected the answer option that they would order one. Subsequently, the participants were asked to select which imaging modality they considered most suitable to diagnose or exclude the presence of posttraumatic osteomyelitis (more than one answer was allowed). The imaging options given were: ultrasound, ultrasound-guided biopsy, CT-scan with or without intravenous contrast, CT-scan-guided biopsy, MRI scan with or without intravenous contrast, 3-phase bone scan with or without SPECT/CT, white blood cell scintigraphy with or without SPECT/CT, and FDG-PET with or without CT. There was also the possibility to provide a personal, non-listed answer.

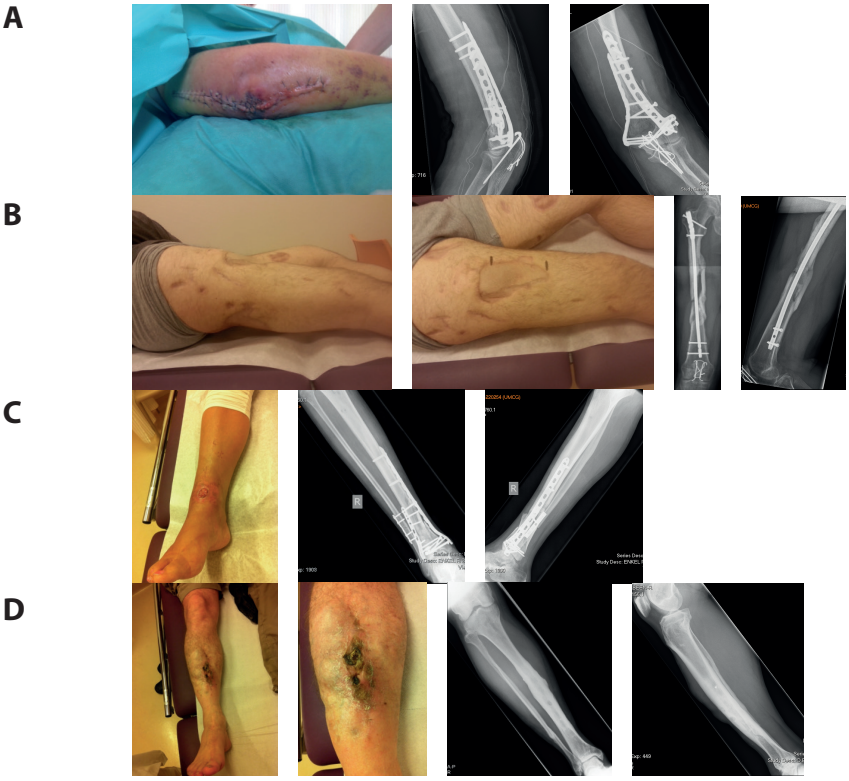

Figure 1. Patient-based clinical scenarios. Patient **A:** A 58-year-old healthy man underwent an open reduction and internal fixation (ORIF) of a comminuted intra-articular distal humerus fracture one week ago. The postoperative X-Ray showed an adequate fracture reduction and good position of the metalwork. After one week a wound infection was diagnosed and it was decided to bring the patient back to theatre for a wound washout. His CRP is 68 mg/l , white cell count 11.5x10^9/l. Patient **B:** A 23-year-old healthy man underwent intramedullary nailing for a Gustillo grade 3B open comminuted femur fracture one year ago. The initial stabilization was followed by multiple wound debridements, changing of Vacuum Assisted Closure (VAC) dressings and finally split skin grafting of the wound. During the repeated VAC changes the nail was palpable in the wound. The patient's main complaint is pain around the fracture site over the last few months. On examination there is no wound breakdown. His CRP is 27 mg/l, white cell count 6.5x10^9/l and ESR 48 mm/hr. Patient **C:** A 44-year-old healthy woman underwent ORIF of a closed comminuted distal tibia fracture five months ago. She is referred because the operation wound broke down two weeks postoperatively and has not healed since. Her CRP is 3.3 mg/l, white cell count 8.1x10^9/l. Patient **D:** A 49-year-old healthy man underwent multiple operations because of an open fracture of his right tibia and fibula 30 years ago. Although the treatment was complicated by a deep surgical site infection, bone healing was eventually achieved and all metalwork was removed a few years after his last operation. The wound settled down until 18 months ago, when an unstable crust developed in the scar. His CRP is 5.9 mg/l, white cell count 8.9 x 10^9/l. Question after each case description: a) Would you order a conventional X-Ray? (Note: X-Rays are only provided if the responder selected 'yes'). b) Would you request further imaging? If yes: please select preferred imaging modality (more than one answer possible, please see the materials and methods section for details).

Participants were also asked which serum inflammatory marker they thought was specific enough to be used for diagnosing PTO (CRP, LC or ESR) and whether they were aware of a local hospital protocol for diagnosis and management of PTO.

Data analysis

Data were analysed using IBM SPSS Statistics for Windows (version 22.0, Armonk, NY: IBM Corp.).

RESULTS

The overall response rate was 15% (n= 346); 27% of the trauma surgeons (n=155), 8% of the orthopaedic surgeons (n= 102), 35% of the nuclear medicine physicians (n= 57) and 12% of the MSK radiologists (n=33) responded. Table 1 presents the responders' characteristics.

Table 1. Responders' characteristics.

	Trauma surgeon (N=153)	Orthopedic surgeon (N=104)	Nuclear physician (N=56)	MSK radiologist (N=33)
Age (years)				
< 35	30 (20)	23 (22)	7 (12)	8 (24)
35–50	89 (58)	53 (51)	34 (61)	13 (39)
> 50	34 (22)	28 (27)	15 (27)	12 (36)
Medical experience				
Registrar	32 (21)	21 (20)	0 (0)	4 (12)
Consultant	121 (79)	83 (80)	56 (100)	29 (88)
Hospital type				
Non-teaching hospital (n=97)	22 (14)	40 (38)	31 (55)	10 (30)
Peripheral teaching hospital (n=28)	79 (52)	34 (33)	11 (20)	11 (33)
University teaching hospital (n=8)	52 (34)	30 (29)	14 (25)	12 (36)

Data are presented as N (%).

The results for the preferred medical imaging modalities for patients A–D are listed in Table 2. There was consensus on the usefulness of a conventional X-Ray in patients with a late infection (patient B, C and D). In patient A with an early SSI a repeat X-Ray was requested only 203 times (54.4%); in patients B, C and D a conventional X-Ray was requested respectively 363 (97.3%), 357 (95.7%) and 351 (94.1%) times.

In those patients with late PTO (B, C and D) there was a remarkable difference between trauma surgeons and orthopaedic surgeons in terms of choice of nuclear medicine imaging. Trauma surgeons favoured FDG-PET, while orthopaedic surgeons preferred the WBC scintigraphy. A similar, consistent difference was seen between radiologists and nuclear medicine physicians regarding the choice for radiology imaging versus nuclear medicine imaging. For example, in patient D an MRI was favoured by 82% of the MSK radiologists versus 2% of the nuclear medicine physicians. For an FDG-PET/CT for the same patient these percentages were 3% versus 36% respectively. CT-scans and 3-phase bone scans for late fracture-related infections were popular among orthopaedic surgeons and to a lesser extent MSK radiologists, though not by trauma surgeons or nuclear medicine physicians. Ultrasound-guided biopsy was regarded by all physicians to have some role in patients with an early infection (patient A), but was not popular for patients with late infections.

Table 2. Responders' preferred imaging modalities per patient scenario.

	X-Ray	Ultrasound	Ultrasound-guided biopsy	CT-scan *without* IV contrast	CT-scan *with* IV contrast	CT-guided biopsy	MRI scan *without* IV contrast	MRI scan *with* IV contrast	3-phase bone scan *with/without* SPECT/CT	WBC scintigraphy *with/without* SPECT/CT	FDG-PET *with/without* CT
Patient A											
Trauma surgeon (N=153)	88 (58)	4 (3)	8 (5)	0 (0)	1 (1)	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)
Orthopaedic surgeon (N=104)	65 (63)	11 (11)	14 (14)	2 (2)	1 (1)	0 (0)	0 (0)	2 (2)	1 (1)	2 (2)	0 (0)
Nuclear physician (N=56)	20 (36)	16 (29)	6 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	10 (18)
MSK radiologist (N=33)	21 (64)	13 (39)	12 (36)	1 (3)	4 (12)	0 (0)	0 (0)	14 (42)	4 (12)	6 (18)	3 (9)
Patient B											
Trauma surgeon (N=153)	153 (100)	1 (1)	3 (2)	2 (1)	6 (4)	2 (1)	0 (0)	2 (1)	5 (3)	3 (2)	49 (32)
Orthopaedic surgeon (N=104)	102 (98)	3 (3)	5 (5)	36 (35)	9 (9)	8 (8)	2 (2)	6 (6)	23 (22)	33 (32)	8 (8)
Nuclear physician (N=56)	48 (86)	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (9)	2 (4)	14 (25)
MSK radiologist (N=33)	33 (100)	1 (3)	1 (3)	5 (15)	4 (12)	0 (0)	1 (3)	17 (52)	10 (30)	7 (21)	7 (21)
Patient C											
Trauma surgeon (N=153)	151 (99)	0 (0)	0 (0)	6 (4)	5 (3)	0 (0)	0 (0)	2 (2)	4 (3)	3 (2)	41 (27)
Orthopaedic surgeon (N=104)	103 (99)	3 (3)	2 (2)	37 (36)	9 (9)	6 (6)	5 (5)	6 (6)	26 (25)	23 (22)	8 (8)
Nuclear physician (N=56)	46 (82)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (9)	2 (4)	15 (27)
MSK radiologist (N=33)	32 (97)	3 (9)	2 (6)	4 (12)	4 (12)	0 (0)	0 (0)	17 (52)	10 (30)	8 (25)	6 (18)
Patient D											
Trauma surgeon (N=153)	146 (95)	4 (3)	1 (1)	2 (1)	3 (2)	1 (1)	0 (0)	5 (3)	4 (3)	3 (2)	46 (30)
Orthopaedic surgeon (N=104)	104 (100)	2 (2)	1 (1)	12 (12)	10 (10)	6 (6)	15 (14)	41 (39)	26 (25)	23 (22)	8 (8)
Nuclear physician (N=56)	44 (79)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	5 (9)	0 (0)	20 (36)
MSK radiologist (N=33)	31 (94)	1 (3)	1 (3)	3 (9)	0 (0)	0 (0)	1 (3)	27 (82)	11 (33)	1 (3)	1 (3)

Data are presented as N(%).

The choice for an imaging modality was also influenced by the availability of this technique in the responder's own hospital (Table 3). For example, of all those responders who could perform a FDG-PET/CT in their own institution, 21.2% elected it as their preferred imaging method of choice, whereas it was chosen by only 7.2% of responders who did not have a FDG-PET/CT in their own hospital available.

Table 3. Responder's preferred imaging modalities per patient scenario corrected for the in hospital available imaging techniques.

	X-Ray	Ultrasound	Ultrasound-guided biopsy	CT-scan *without* IV contrast	CT-scan *with* IV contrast
Patient A					
Trauma surgeon	88 (56) (N=152)	4 (3) (N=151)	8 (5) (N=151)	0 (0) (N=150)	1 (1) (N=150)
Orthopaedic surgeon	65 (63) (N=103)	10 (10) (N=98)	14 (14) (N=98)	2 (2) (N=96)	1 (1) (N=96)
Nuclear physician	18 (33) (N=54)	16 (30) (N=54)	6 (11) (N=54)	0 (0) (N=55)	0 (0) (N=55)
MSK radiologist	21 (64) (N=33)	13 (39) (N=33)	12 (36) (N=33)	1 (3) (N=33)	4 (12) (N=33)
Patient B					
Trauma surgeon	152 (100) (N=152)	1 (1) (N=151)	3 (2) (N=151)	2 (1) (N=150)	6 (4) (N=150)
Orthopaedic surgeon	101 (98) (N=103)	2 (2) (N=98)	4 (4) (N=98)	34 (35) (N=96)	8 (8) (N=96)
Nuclear physician	47 (87) (N=54)	2 (4) (N=54)	2 (4) (N=54)	0 (0) (N=55)	0 (0) (N=55)
MSK radiologist	33 (100) (N=33)	1 (3) (N=33)	1 (3) (N=33)	5 (15) (N=33)	4 (12) (N=33)
Patient C					
Trauma surgeon	150 (99) (N=152)	0 (0) (N=151)	0 (0) (N=151)	6 (4) (N=150)	5 (3) (N=150)
Orthopaedic surgeon	102 (99) (N=103)	2 (2) (N=98)	1 (1) (N=98)	36 (38) (N=96)	8 (8) (N=96)
Nuclear physician	44 (82) (N=54)	1(2) (N=54)	1 (2) (N=54)	0 (0) (N=55)	0 (0) (N=55)
MSK radiologist	32 (97) (N=33)	3 (9) (N=33)	2 (6) (N=33)	4 (12) (N=33)	4 (12) (N=33)
Patient D					
Trauma surgeon	145 (95) (N=152)	4 (3) (N=151)	1 (1) (N=151)	2 (1) (N=150)	3 (2) (N=150)
Orthopaedic surgeon	103 (100) (N=103)	1 (1) (N=98)	1 (1) (N=98)	12 (13) (N=96)	9 (9) (N=96)
Nuclear physician	42 (78) (N=54)	2 (4) (N=54)	0 (0) (N=54)	0 (0) (N=55)	0 (0) (N=55)
MSK radiologist	31 (94) (N=33)	1 (3) (N=33)	1 (3) (N=33)	3 (9) (N=33)	0 (0) (N=33)

Data are presented as number of responders n (%) who would select this imaging modality compared to the total number of responders (N) with this imaging modality available in their hospital.

None of the serum inflammatory markers was regarded as very specific for diagnosing PTO, but CRP was thought to be the most useful laboratory test and the most popular amongst orthopaedic surgeons (Table 4). One-third of all responders (36%, n=124) reported being aware of a hospital protocol for the treatment of osteomyelitis, the other responders were either unaware of a protocol (25%, n=86) or reported an absence of one (39%, n=136) (table 5). The availability of a PTO protocol was highest in the University Medical Centres (Table 6).

CT-guided biopsy	**MRI scan *without* IV contrast**	**MRI scan *with* IV contrast**	**3-phase bone scan *with/without* SPECT/CT**	**WBC scintigraphy *with/without* SPECT/CT**	**FDG-PET *with/ without* CT**
1 (1) (N=150)	0 (0) (N=148)	1 (1) (N=148)	0 (0) (N=19)	0 (0) (N=15)	1 (1) (N=103)
0 (0) (N=96)	0 (0) (N=96)	2 (2) (N=96)	1 (1) (N=83)	2 (3) (N=72)	0 (0) (N=54)
0 (0) (N=55)	0 (0) (N=52)	0 (0) (N=52)	1 (17) (N=6)	0 (0) (N=6)	10 (21) (N=48)
0 (0) (N=33)	0 (0) (N=33)	14 (42) (N=33)	4 (13) (N=30)	6 (32) (N=19)	3 (12) (N=25)
2 (1) (N=150)	0 (0) (N=148)	2 (1) (N=148)	5 (26) (N=19)	2 (13) (N=15)	38 (37) (N=103)
8 (8) (N=96)	2 (2) (N=96)	6 (6) (N=96)	20 (24) (N=83)	28 (39) (N=72)	7 (13) (N=54)
0 (0) (N=55)	0 (0) (N=52)	0 (0) (N=52)	4 (67) (N=6)	2 (33) (N=6)	13 (27) (N=48)
0 (0) (N=33)	1 (3) (N=33)	17 (52) (N=33)	10 (33) (N=30)	6 (32) (N=19)	7 (28) (N=25)
0 (0) (N=150)	0 (0) (N=148)	2 (2) (N=148)	4 (21) (N=19)	1 (7) (N=15)	33 (32) (N=103)
6 (6) (N=96)	5 (5) (N=96)	6 (6) (N=96)	23 (28) (N=83)	19 (26) (N=72)	7 (13) (N=54)
0 (0) (N=55)	0 (0) (N=52)	0 (0) (N=52)	5 (83) (N=6)	2 (33) (N=6)	14 (29) (N=48)
0 (0) (N=33)	0 (0) (N=33)	17 (52) (N=33)	11 (37) (N=30)	7 (37) (N=19)	6 (24) (N=25)
1 (1) (N=150)	0 (0) (N=148)	5 (3) (N=148)	5 (26) (N=19)	1 (7) (N=15)	37 (36) (N=103)
6 (6) (N=96)	15 (16) (N=96)	40 (42) (N=96)	29 (35) (N=83)	20 (28) (N=72)	7 (13) (N=54)
0 (0) (N=55)	0 (0) (N=52)	1 (2) (N=52)	4 (67) (N=6)	0 (0) (N=6)	17 (35) (N=48)
0 (0) (N=33)	1 (3) (N=33)	27 (82) (N=33)	4 (13) (N=30)	0 (0) (N=19)	1 (4) (N=25)

Table 4. Preferred serum inflammatory markers for diagnosing PTO.

	C-reactive protein	Leukocyte count	Erythrocyte sedimentation rate
Trauma surgeon (N=153)	86 (56)	47 (31)	63 (41)
Orthopaedic surgeon (N=104)	74 (71)	26 (25)	55 (53)
Nuclear medicine physician (N=56)	29 (52)	20 (36)	16 (29)
MSK radiologist (N=33)	14 (42)	14 (42)	12 (36)

Data are presented as N(%). Result of the question: 'Which serum inflammatory marker do you regard useful for diagnosing PTO'? Note: more than one answer was possible.

Table 5. Availability of PTO protocol per medical specialty.

Medical specialty		Frequency	Percent
Trauma surgeon (N=153)	yes	51	33
	no	71	46
	unsure	31	20
Orthopedic surgeon (N=104)	yes	47	45
	no	41	39
	unsure	16	15
Nuclear medicine physician (N=56)	yes	13	23
	no	15	27
	unsure	28	50
MSK radiologist (N=33)	yes	13	39
	no	9	27
	unsure	11	33

Table 6. Availability of PTO protocol per hospital type.

Type of hospital		Frequency	Percent
Non-teaching hospital (N=103)	yes	23	22
	no	50	49
	unsure	30	29
Teaching hospital (N=35)	yes	45	33
	no	59	44
	unsure	31	23
University medical centre (N=8)	yes	56	52
	no	27	25
	unsure	25	23

DISCUSSION

This study confirms the variety in diagnostic strategies that many clinicians dealing with PTO will recognise from their day-to-day practice. Although the overall response rate of our survey was only 15%, the responders are a typical reflection of those working with this patient group (Table 1). One should also keep in mind that it is only a small percentage of all trauma and orthopaedic surgeons who are involved in osteomyelitis care and that we addressed the whole group. Because it is likely that the responders will have an interest in – and therefore deeper knowledge of – PTO compared to non-responders, this study is prone to even underestimate the real variety in diagnostic imaging strategies as the first diagnostic manoeuvres might be initiated by the primary surgeon. We therefore regard the contribution from 346 medical practitioners as a substantial response and the outcome of this survey as a relevant finding to report to our peers.

The variation in diagnostic workup of patients with suspected PTO is in concordance with the lacking guidelines on this subject and also with the apparent struggle of various authors to formulate clear and practical recommendations. Termaat et al. published a meta-analysis on optimal imaging modalities for chronic osteomyelitis [18]. They concluded that FDG-PET was the most accurate imaging option to diagnose chronic osteomyelitis, with a sensitivity and specificity of 96% and 91% respectively. However, the paper was published in 2005 and includes studies published between 1975 and 2003. Considering that current medical technology is developing at an almost exponential rate it is safe to assume that the diagnostic capacities of the different imaging modalities described are no longer truly represented by the papers analysed for that study (e.g. the commercial system to combine PET with CT (PET/CT) first reached the market in 2001 [26]). The data in that paper should therefore be interpreted cautiously. Also based on the best-available evidence, but still a result from a consensus meeting, is the report of the European Association of Nuclear Medicine (EANM) published in 2014. In that paper Jutte et al. proposed a diagnostic flowchart for peripheral bone osteomyelitis, including sternal infections [14]. This flowchart is probably the best available tool for clinicians at the moment, but it is a very broad algorithm with an emphasis on nuclear imaging. In the present study, recommendations of this EANM consensus document were not followed by the majority of responders in any of the scenarios presented.

Part of the variance in diagnostic imaging strategies for PTO can be explained from the imaging techniques locally available to the requesting (or advising) medical practitioner (Table 3). Responders tended to favour an imaging modality when this was available in their hospital. Although this is an understandable pragmatic choice, it may not be

the most cost-effective strategy. Having an evidence-based guideline for diagnosing (and excluding) PTO will support a radiology and/or nuclear medicine department in negotiating the purchase of future appropriate medical imaging equipment.

Yet another possible explanation for the variance between the subgroups is that, in the Netherlands, the majority of fractures are treated by trauma surgeons who are trained as general surgeons, as opposed to orthopaedic surgeons (66% versus 34% respectively as reported in a recent study on hip fractures [27]). Orthopaedic surgeons are more familiar with the (more researched) concept of prosthetic joint infections (PJI), and some of their choices for diagnosing PTO might be extrapolated from these papers. Dutch trauma surgeons however focus solely on fractures and are not influenced by previous knowledge on diagnosing PJI, therefore they might have a different approach to diagnosing fracture-related infections. The same can be said for radiologists versus nuclear medicine physicians – both are highly trained in medical imaging options for various infectious conditions, but they are likely biased by background knowledge of their own area of expertise. This bias does explain the difference in preferred imaging modalities in patients with late-onset PTO.

Also, surgical clinicians and advising imaging specialists often have a different starting point when additional imaging has to be chosen. The clinical situation plays a crucial role in the decision making process and nuclear imaging specialists and radiologists have the disadvantage of not being able to examine patients themselves. In this study the provided clinical patient scenario's were the same for all participating medical specialists but the difference in background knowledge might have lead to a different imaging strategy. More in general, failure from the surgeon to communicate the essential clinical details and specific diagnostic question with the advising imaging specialist can result in a less logical imaging advice. Another important factor that needs to be emphasised is that the process of treating fracture-related infections is time-consuming and costly. The best available data for this is derived from studies in patients with a prosthetic joint infection (PJI) and diabetic feet. In infected total hip arthroplasties (THAs), for example, the hospital length of stay has been shown to be 2.2 times longer, with associated overall costs 3.1 times higher compared to non-infected primary THA procedures [28, 29]. Non-medical costs resulting from the inability to work and help required from carers are not known. Any delay in diagnosis will obviously also delay the start of treatment and subsequently the recovery of a patient with PTO, hence overall costs will increase. It is known from other orthopaedic studies that, as a general rule, both patients and cost-effectiveness benefit from clinical pathways and guidelines [30, 31]. There is thus a need for a lean and strict algorithm on diagnosing PTO. This will help clinicians choose the most effective diagnostic pathway in order to

reduce the time needed to properly diagnose PTO and subsequently reduce medical costs by avoiding unnecessary imaging requests. Our results lead us to believe that in some cases a leaner diagnostic pathway could have been followed. For example, for the two patients with a late clinical wound breakdown and therefore a clear infective component (patient C and D), 44% (patient C, n=152) and 43% (patient D, n=149) of all participants would request further imaging, which is mainly indicated to diagnose or exclude an infection (a bone scan, WBC scintigraphy or FDG-PET). Especially for patient D (an obvious infection, no hardware in situ and all operations performed three decades ago), one could argue that it is more logical to request an imaging modality that will aid in determining the surgical strategy and not only confirm the diagnosis of osteomyelitis. An MRI scan to visualise the extent of the osteomyelitis and the presence or absence of cloacae, sinuses, subcortical abscesses and intramedullary sequesters would in this case be a more logical option and is much cheaper and easier to perform than, for example, a WBC scintigraphy or FDG-PET. It is in this perspective interesting to note that an MRI for patient D was selected by only 26% (n=90) of the responders.

The present study was designed to assess current practice on diagnostic imaging strategies for posttraumatic osteomyelitis in The Netherlands. The results will be used as a baseline for the development of a multicentre prospective trial to eventually provide and implement evidence-based national and international guidelines on diagnosing PTO. These guidelines will hopefully decrease the time to diagnosis in a cost-effective way.

Limitations of this study

This study might be limited due to bias resulting from under-coverage and non-response. Because it is likely that the responders will have an interest in – and therefore deeper knowledge of – PTO compared to non-responders, this study is prone to underestimating the real variety in diagnostic imaging strategies. A second limitation might be the fact that this study was undertaken in only one European country. However since no international guidelines on this topic exist it is likely that the lacking consensus on how to diagnose PTO is an international omission and that our results can be extrapolated to other trauma orthopaedic societies.

CONCLUSIONS

There is no agreement amongst Dutch trauma and orthopaedic surgeons, radiologists and nuclear physicians regarding the optimal diagnostic strategies to diagnose or exclude posttraumatic osteomyelitis. None of the serum inflammatory markers was

regarded as very specific for diagnosing PTO, but CRP was thought to be the most useful laboratory test. The availability of and awareness towards local protocols to diagnose and treat PTO is poor. The results of this study support the need for future randomised controlled trials on optimal diagnostic strategies for PTO. There is also a necessity for the development of national and international guidelines on this topic, in which cost effective strategies are based on the best available evidence.

Compliance with ethical standard

Conflict of interest: Geertje Govaert, Andor Glaudemans, Joris Ploegmakers, Alain Viddeleer, Klaus Wendt and Inge Reininga declare that they have no conflict of interest in connection with this article.

Ethical standards: Given the nature of this study, a waiver for ethical assessment was granted by the hospital medical ethical committee of the University Medical Centre Groningen (METc2014.554). Written informed consent was obtained from all patients for the use of their clinical and medical imaging.

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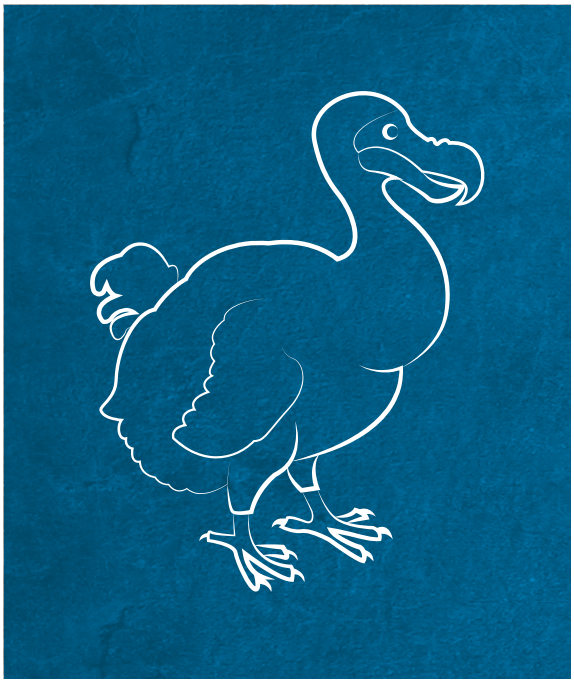

PART II

MEDICAL IMAGING

CHAPTER 3

Nuclear medicine imaging of posttraumatic osteomyelitis

G.A.M. Govaert, A.W.J.M. Glaudemans

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ABSTRACT

Early recognition of a possible infection and therefore a prompt and accurate diagnostic strategy is essential for a successful treatment of posttraumatic osteomyelitis (PTO). However, at this moment there is no single routine test available that can detect osteomyelitis beyond any doubt and the performed diagnostic tests mostly depend on personal experience, available techniques and financial aspects. Nuclear medicine techniques focus on imaging pathophysiological changes which usually precede anatomical changes. Together with recent development in hybrid camera systems, leading to better spatial resolution and quantification possibilities, this provides new opportunities and possibilities for nuclear medicine modalities to play an important role in diagnosing PTO.

In this overview paper the techniques and available literature results for PTO are discussed for the three most commonly used nuclear medicine techniques: the three phase bone scan (with SPECT-CT), white blood cell scintigraphy (also called leukocyte scan) with SPECT-CT and ^{18}F-fluorodeoxyglucose (FDG)-PET/CT. Emphasis is on how these techniques are able to answer the diagnostic questions from the clinicians (trauma and orthopaedic surgeons) and which technique should be used to answer a specific question. Furthermore, three illustrative cases from clinical practice are described.

INTRODUCTION

Osteomyelitis covers a wide range of bone infections caused by an infecting organism. Normally, bone is resistant to bacterial colonization; in trauma however the bone integrity can be disrupted by fractures, surgery or the presence of metal implants which makes it more vulnerable to exogenous microbial invasion. This, combined with the typically acute setting in which trauma surgery takes place with possibly contaminated open fractures, soft tissue injury and hasty damage control procedures, leads to a reported incidence of 1 to 19 % of deep infections after surgical fracture care. Not only this high infection rate is a concern but also due to an increase in surgical procedures over the last decades, fracture related osteomyelitis, also referred to as posttraumatic osteomyelitis (PTO), becomes more and more an entity that trauma - and orthopaedic surgeons will have to deal with [1,2].

Essential for a successful treatment of PTO is an early recognition of the possible infection and therefore a prompt and accurate diagnostic strategy. A surgical site infection (SSI) occurs in the early phase (first 2 weeks after surgery) and can usually be recognized by clinical examination, since mostly the well-known four signs of an infection (swelling, redness, pain and heat) are present. In the later phases of PTO these signs may not be present and diagnosis can be difficult. It is however of invaluable importance to diagnose PTO as early as possible and to start early and specific treatment, since a late recognition or inadequate treatment may result in prolonged disease duration, high recurrence rate, high morbidity and sometimes even an amputation [3].

The diagnostic problem in PTO is that there is no single routine test available that can detect an infection with sufficiently high diagnostic accuracy. Mostly, a combination of clinical, laboratory, microbiological and medical imaging tests is performed [4] and the followed strategy depends on personal experience, tradition, financial aspects of the institute and best available evidence.

Diagnostic imaging routinely performed consists of plain X-rays and computed tomography (CT). These techniques are helpful to assess the position of metal implants and the union rate of the fracture, but are not able to differentiate between infection and inflammation. Magnetic resonance imaging (MRI) is better able to recognize infections; however, the metal implants can introduce artefacts and its diagnostic accuracy decreases after recent surgery as differentiation between sterile inflammation and infected tissue is difficult [5-7]. To our opinion, nuclear medicine imaging techniques plays an important role in the diagnostic pathway to diagnose PTO. Nuclear medicine, which focusses at the pathophysiology of processes, is a booming

area within the medicinal community. Pathophysiological changes usually precede anatomical changes, often leading to an earlier and possibly more accurate diagnosis. Recent developments in hybrid camera systems, combining the best of both anatomy and physiology with higher spatial resolution and better quantification possibilities, provides new opportunities and possibilities for these hybrid imaging modalities to play an important role in both diagnosis and therapy evaluation in patients with PTO.

The aim of this paper is to explain the existing nuclear medicine imaging possibilities for diagnosing PTO, how these modalities are able to answer the diagnostic questions from the clinicians (trauma –and orthopaedic surgeons) and to provide an overview of which nuclear imaging technique should be used at which time point of the diagnostic pathway.

Nuclear medicine in general; SPECT and PET

In nuclear medicine, radiopharmaceuticals (a radioactive element attached to a chemical compound or pharmaceutical specific for a disease process) are administered intravenously into the patient. As a result, images are performed from radiation which is emitted at the location of the disease/infectious process from within the patient. This characteristic forms the main distinction with radiology, which mainly focuses on tissue anatomy by using external radiation sources.

The two main camera systems used in nuclear medicine to visualize the radiopharmaceuticals are the gamma camera and the PET camera (Figure 1). These camera systems detect the γ-rays emitted from the patient and transform it into an image (planar and/or 3D).

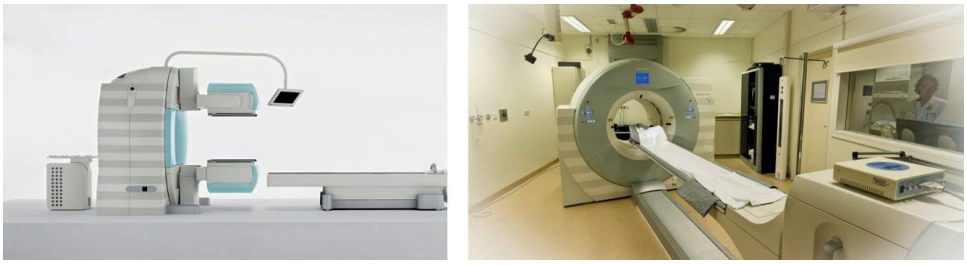

Figure 1. Left image: gamma camera with SPECT-CT possibility (Siemens Symbia T). Right: PET-CT camera (Siemens Biograph mCT 64-slice).
Image courtesy: Siemens Medical Systems, Knoxville, TN.

The already since the 1970s existing *gamma camera* forms the basis of conventional nuclear medicine by providing 2D planar imaging of the body. However, this technique has several limitations: image quality is rather poor and the spatial resolution of the gamma camera is limited to approximately 8 mm. Furthermore, it is difficult, based on 2D images with overlapping structures, to determine exactly where the increased uptake is located. The effect of this superposition can be overcome by collecting images from different angles (64 or 128) around the patient, thereby creating a 3D image. This technique, called *single photon emission computed tomography* (*SPECT*), leads to a higher contrast and improves sensitivity.

Positron emission tomography (*PET*) is a more recently (1990s) developed unique imaging tool to visualize various pathophysiological processes in the body. This technique is based on radionuclides that emit positrons (positively charged electrons) to become stable. Emitting positrons cannot exist freely, and therefore it meets his antimatter and annihilates into two γ-ray photons, each with the same energy and moving in opposite directions. The PET camera exists of a ring-shaped detector system which can detect the two photons when arriving within a certain time frame at opposite detectors. Recent developments in software lead to a correction method for the time a photon needs to travel from its origin to the detector. This software development, called time-of-flight (TOF) has major advantages for spatial resolution.

The major advantage of PET above SPECT is that that PET camera system has a greater efficacy in detecting photons, and a better spatial resolution of around 3-4 mm. Furthermore, quantification possibilities are better with PET.

Added value of hybrid imaging; SPECT/CT and PET/CT

As already mentioned earlier, recent development in both soft- and hardware led to the implementation of hybrid systems, combining SPECT and PET with CT. Both the nuclear medicine and radiological technique are performed in an immediate sequential setting, without changing the position of the patients, leading to an almost perfect correlation of pathophysiological with anatomical information. Furthermore, costs are reduced (one imaging modality), and the one-stop-shop principle (one combined scan instead of two separate scans at two different departments) reduces waiting time for the patient.

Very recently, PET systems were also combined with MRI, thereby introducing the PET/MRI hybrid imaging system. In these PET/MRI systems, the different modalities can be used in a simultaneous setting. PET/MRI has several major theoretical advantages that

could be of interest for the whole medical community [8]. At the moment, this modality is mainly used in neurology and cardiology and its role in infectious processes in the musculoskeletal system has to be established.

Nuclear medicine techniques to image PTO

Many radiopharmaceuticals are available to image infectious and inflammatory processes [9]. Only the worldwide most commonly used nuclear medicine techniques to image PTO will be discussed here by explaining the technical details of the procedure supplemented with a brief overview of the relevant literature. Finally, we will provide some illustrative clinical examples.

Bone scintigraphy

Technique

The bone scintigraphy is one of the oldest existing nuclear medicine techniques and still one of the cornerstones in nuclear medicine practice. Radiopharmaceuticals used for bone scintigraphy are diphosphonates coupled to the radionuclide Technetium-99m (^{99m}Tc). These bone-seeking radiopharmaceuticals selectively accumulate on the surface on bone mineral matrix in areas of high metabolic activity and therefore depict osteoblastic activity.

When a musculoskeletal infection is suspected, a three phase bone scintigraphy can be performed as a first screening tool (Figure 2). As revealed by its name, this bone scintigraphy consists of three phases. The first phase is the perfusion phase, or flow study, performed dynamically, over the part of interest, for the first two minutes after administration of the radiopharmaceutical. The second phase is the blood pool phase, also performed on the part of interest, directly after the first phase (2-5 minutes after injection). The third phase, also called the static phase, depicts the incorporation of the radiopharmaceutical into the matrix of the bone and is usually performed 3 hours after administration. This late phase can be combined with a SPECT-CT to localize the area(s) of increased bone metabolism. All three phases are necessary in cases of suspected bone infection, since the three phases characterizes both the vascularization and the metabolic activity of a process.

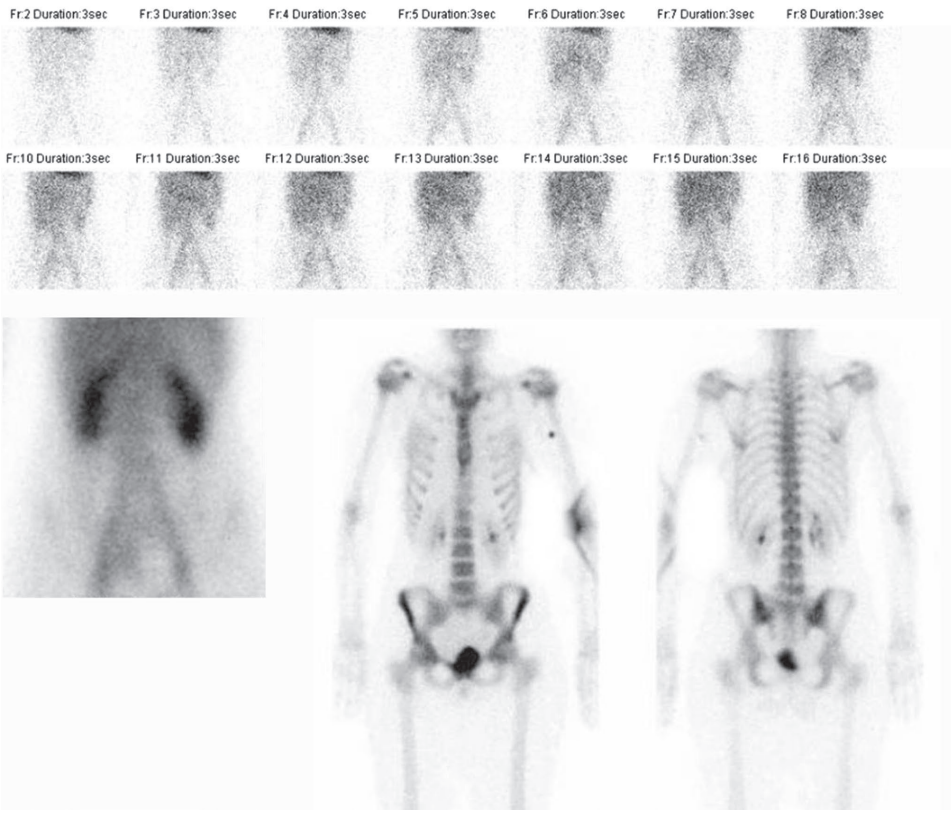

Figure 2. Example of a normal three phase bone scan in a patient with pain complaints of the lumbar spine. Upper row images: flow/perfusion images (phase 1). Lower row, left image: blood pool image (phase 2). Lower row, middle image (anterior view, phase 3), right image (posterior view, phase 3).

Bone scintigraphy in PTO

The three phase bone scan can be used as a first screening method for diagnosing PTO. Because of its good availability it can mostly be performed short (< 24 hours) after the request of the referring clinician and it is relatively cheap. A normal bone scan (no increased perfusion and blood pool, no uptake in the late phase) rules out almost completely an existing bone infection (high sensitivity). The role of the bone scintigraphy however in the acute setting is neglectable, since the specificity is rather low and uptake is visible in all sites of increased bone metabolism irrespective of the underlying cause. A positive bone scan with an increased vascularity and increased metabolic uptake may indicate PTO; yet it can also indicate healing fracture(s) or a postsurgical situation. Furthermore, in a low-grade infection even the first two phases can be negative, so the

late phase is essential and when positive it may be the only indication of an infection. Literature studies trying to find out at which time point a bone scan becomes negative after fractures and/or surgery are scarce. It is known that a bone scan may be positive for at least 2 years after total hip arthroplasty (THA) and 5 years after total knee arthroplasty (TKA) due to physiological bone remodeling after implantation [5]. We do not know exactly the time frame in which the bone scan is definitely positive following trauma, fracture or after open reduction and internal fixation (ORIF) of a fracture. Probably this time period will be around 1-2 years.

In conclusion, there is no role for a bone scintigraphy for diagnosing an SSI or early PTO. There is probably a role (when negative it excludes an infection) in the long-standing PTO, but a positive bone scintigraphy must be interpreted with caution and other imaging methods are necessary to differentiate between an infection and other causes of increased osteoblastic activity.

The "better" bone scan

The conventional bone scan as mentioned above is still the gold standard in bone imaging. The images are acquired on a gamma camera and most new camera systems have also the possibility to include SPECT-CT in the imaging process. However, there is also a PET tracer for bone imaging which uses the radiopharmaceutical ^{18}F-sodium fluoride (^{18}F-NaF). The uptake mechanism of ^{18}F-NaF resembles that of ^{99m}Tc-labelled diphosphonates. The faster blood clearance and the twofold higher uptake in developing bone cells of fluoride makes it possible to image faster (1 hour after injection) and lead to better ratios between pathological and physiological bone uptake [10]. The advantage of using this PET tracer is the better resolution and better quantification possibilities. Limitations however are the higher costs and the lower availability worldwide of these techniques, and the non-possibility to perform flow and blood pool imaging. At this moment, the classical bone scan with labelled diphosphonates remains the gold standard when a bone scan is indicated; the ^{18}F-NaF-PET could be considered for the individual patient.

White blood cell (WBC) scintigraphy

Technique

Scintigraphy using labelled autologous white blood cells (WBC scintigraphy or leukocyte scintigraphy) was already developed in the 1970s and is still the gold standard nuclear medicine technique for infections in the musculoskeletal system. It is a specific indicator for leukocyte infiltration into infected bones and soft tissue and is highly specific, since the WBCs accumulate by active migration to the infection. Over time, there have been

major developments in how to correctly acquire, analyze and interpret the images, which eventually led to a high diagnostic accuracy. Also the possibility to better anatomically localize the infection due to the addition of SPECT-CT helped to reach these good results mentioned in the literature.

Despite the high diagnostic accuracy, the whole procedure itself has limitations. First of all, 50 to 100cc of blood has to be collected from the patient. Then, the preparation of the labelled (preferably with ^{99m}Tc-HMPAO) white blood cells is laborious and time consuming (2-3 hours) and must be performed under sterile conditions and strict regulations [11]. Subsequently, the labeled autologous leukocytes are reinjected into the patient. Finally, at least two imaging time points are necessary: 3-4 hours after reinjection and 20-24 hours after reinjection. This dual-time point imaging has to be performed since the accumulation of leukocytes in the infection is a dynamic process: it is the increase in size or intensity in time that is indicating the presence of an infection (Figure 3). When there is a decrease or the uptake is stable in time, then there is no infection but inflammation or physiological bone marrow uptake [8]. This change in uptake in time can be determined visually, but sometimes semi-quantitative evaluation can be a helpful tool as an addition to visual assessment. This is done by calculating ratios between the infectious focus and the contralateral side as background. Again, increase of the ratio in time points to an infection. Due to disintegration of the used radionuclide (^{99m}Tc), the total acquisition time of the late images has to be prolonged accordingly to the half-life of the tracer to establish identical image quality. In accordance with the bone scintigraphy, a SPECT/CT can be performed to exactly localize the leukocyte uptake. The proposed correct procedure of acquisition and analysis of the scans are stated in a recent publication [12].

White blood cell scintigraphy in PTO

The role of WBC scintigraphy in peripheral osteomyelitis is extensively studied. Prandini et al described in a meta-analysis of published papers up to December 2005, in almost 3600 cases, a diagnostic accuracy of 89% [13]. In the included studies however, different acquisition protocols and interpretation criteria were used. Furthermore, SPECT-CT did not exist at that time. So, probably the diagnostic accuracy is even higher when using the correct and standardized protocols and adding the complementary information obtained by SPECT-CT. This was confirmed in two recent retrospective studies using these correct protocols in respectively 61 and 31 patients with peripheral osteomyelitis. The diagnostic accuracy in these studies was found to be very high: 97 and 100% respectively [12, 14].

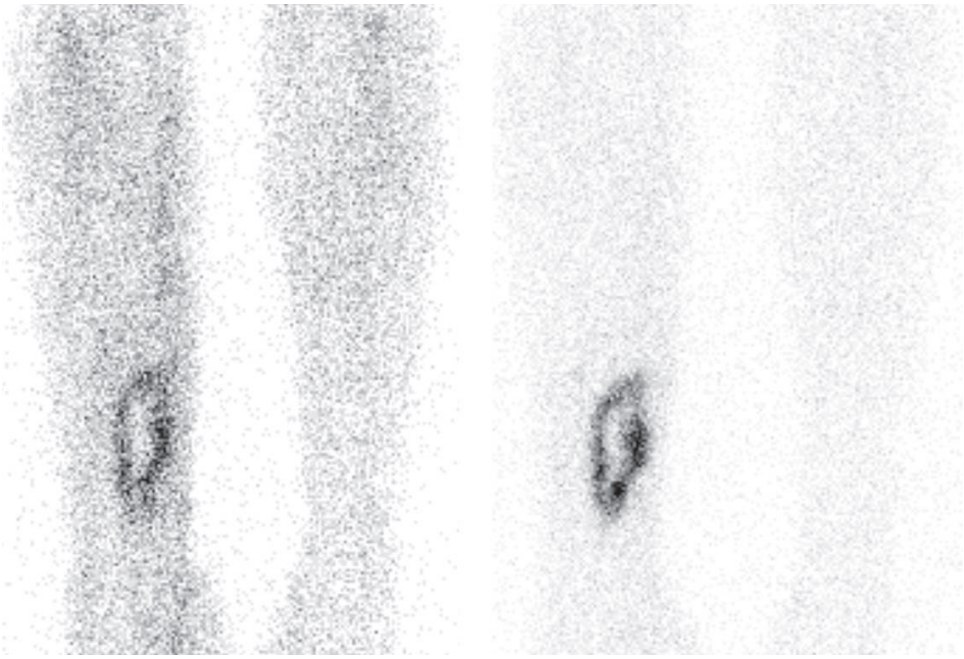

Figure 3. Example of a positive WBC scintigraphy of a 39 year old patient with osteomyelitis of the right tibia. Left image: anterior view 4 hours after injection. Right image: anterior view 24 hours after injection. Increase in uptake in time, especially when the background uptake is taking into account: suspect for an infection.

The difficulty with interpreting the literature on the accuracy of WBC scintigraphy for diagnosing PTO is that most of these studies included patients with peripheral skeletal infections in general (including haematogenous osteomyelitis and prosthetic joint infections). Recently, we performed a systematic review of the recent literature (2000-2015) on the role of imaging modalities in patients with PTO (data not published yet). Only studies were included in which data for at least 10 patients with PTO were available, and a valid reference test (proven by histology or bacteriology, and/or clinical follow-up of more than six months) was described. Unfortunately, only 11 studies could be included of which 4 were performed with WBC scintigraphy (diagnostic accuracies between 61 and 98%). So, despite the extensively available data of WBC scintigraphy in peripheral osteomyelitis in general, there is a lack of studies really focusing on PTO. Despite this disappointed finding, we believe that there is an absolute role for WBC scintigraphy in diagnosing PTO. This is based on expert opinion and best available evidence on osteomyelitis in general since it is the only existing imaging modality that is a specific indicator for an infection. Therefore, we retrospectively reviewed the

diagnostic value of WBC scintigraphy +/- SPECT/CT in 114 patients with suspected PTO in our hospital. Sensitivity, specificity, positive predictive value and negative predictive value were 89, 95, 86 and 97%, respectively (data to be published).

^{18}F-fluorodeoxyglucose (FDG)-PET

Technique

The glucose analogue FDG is already extensively used in oncology for over a decade. It can also be used in infectious diseases, because activated leukocytes, monocytes, lymphocytes, macrophages and giant cells all use glucose as their energy source. To minimize FDG uptake in normal tissue, patients must fast for at least 4-6 hours to reduce competition for glucose transporters on the cell membrane. After the injection of the labelled FDG (^{18}F-FDG) patients must rest for an hour and limit physical activity to minimize muscle uptake and obtain a good biodistribution in the body. High contrast images of infectious lesions can be obtained with this technique. The use of FDG-PET has many advantages: no blood manipulation, high spatial resolution, one imaging time point which is already 60 minutes after injection, one-stop-shop possibility with diagnostic CT etc. It is therefore an essential tool when searching for an infection or inflammation in a patient with fever of unknown origin or to establish the source of dissemination of infectious lesions in the body in a patient with a haematogeneous spread of the infection (Figure 4).

Unfortunately, the uptake in both inflammatory and infectious cells makes this technique also very non-specific as it is often not possible to discriminate between inflammation and infection. No universal accepted interpretation criteria are available to declare a FDG-PET scan positive or negative for an infection, let alone for PTO. Furthermore, uptake of FDG in the peripheral skeleton results in a rather broad differential diagnosis. Osteomyelitis, soft tissue infection, inflammation, granulation tissue after surgery, reactive uptake around foreign body material, atherosclerosis, recent fractures and neuro-osteoarthropathy all lead to increased FDG uptake.

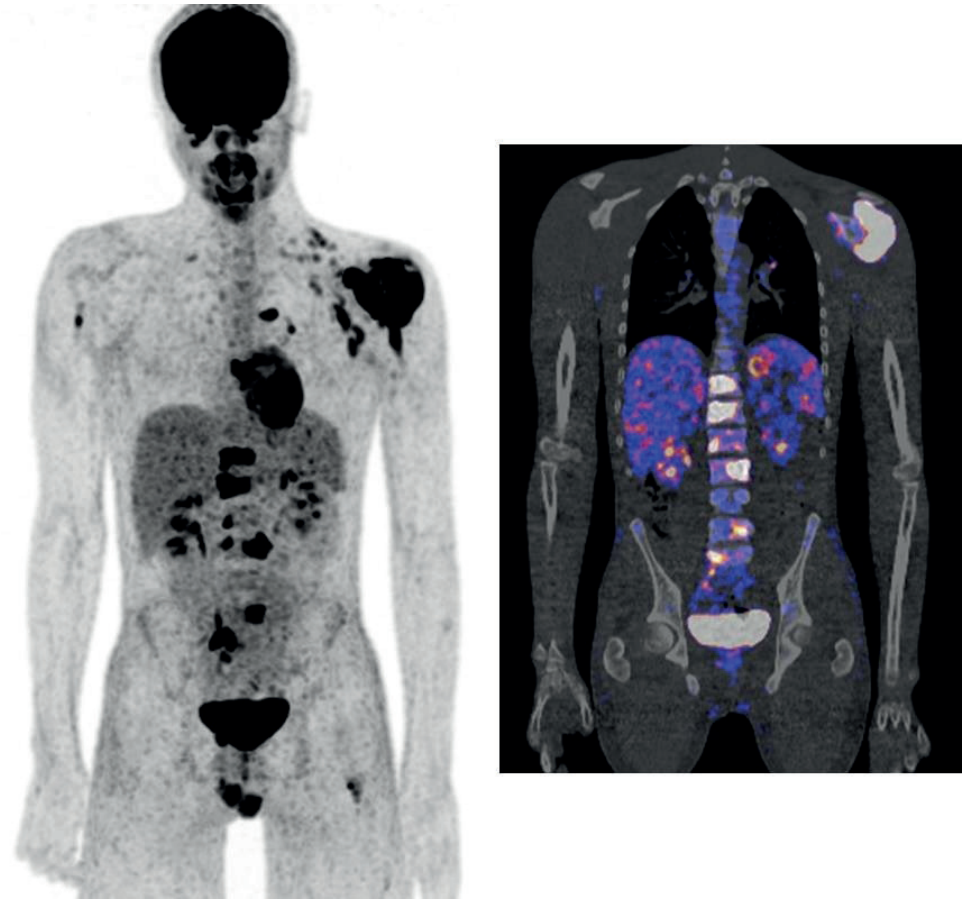

Figure 4. Patient with a proven tuberculosis osteomyelitis of the left shoulder. FDG-PET was performed to identify any disseminated foci of infection. FDG-PET image (left) and fusion PET-CT image (right) showing multiple infectious foci in the body (left shoulder, multiple vertebras, right upper arm, left upper leg).

FDG-PET in PTO

As is the case with WBC scintigraphy, most data are available for FDG-PET imaging in long-standing (chronic) peripheral osteomyelitis. Termaat et al. performed a systematic review and meta-analysis for the assessment of chronic osteomyelitis and found the best results for FDG-PET with a sensitivity of 96% and a specificity of 91% [15]. Similar results were found by Jamar et al., who pooled all available data (in total 287 cases) and found a diagnostic accuracy of 94.5% [16]. However, most of these studies dealt with chronic osteomyelitis from a diverse etiology and the exact role of FDG-PET in the

posttraumatic situation is not known. Theoretically, due to reactive inflammation, the performance may be worse with osteosynthesis in situ, as is the case in prosthetic joint infections.

In the same systematic review our group performed as mentioned earlier, only 5 FDG-PET studies (with or without combined CT scan) were identified that were published in the last 15 years and fulfilled the criteria of > 10 patients with suspected PTO and a valid reference test. The reported diagnostic accuracies were high (between 86 and 95%), but thus far we don't know exactly the percentages of patients with osteosynthesis in situ at time of the scan procedure (data not published yet).

In 2013, a shared guideline for the use of FDG in inflammation and infection was published by both the European Association of Nuclear Medicine (EANM) and the Society of Nuclear Medicine and Molecular Imaging (SNMMI) in the United States. This guideline states that the level of evidence for the use of FDG in osteomyelitis remains low (2b at best), and that at this moment, WBC scintigraphy is the preferred imaging modality. FDG-PET however may be used in the chronic peripheral non postoperative setting [16].

Questions from surgeon's

In daily clinical practice in patients with suspected PTO, the trauma –and orthopaedic surgeons have in general the following questions for the medical imaging specialists:

1. Is the involved bone viable and are there sequestra?

To answer this question, a three phase bone scintigraphy (preferably combined with a SPECT/CT) is required to see if there is perfusion and osteoblastic activity of the involved bone. In the case of suspected PTO, this is the only inquiry that is to be answered by bone scintigraphy. However, as said before: image quality is rather poor and the spatial resolution of the gamma camera is limited to approximately 8 mm, smaller sequestra can therefore be missed. When there is uptake, in the setting of PTO, it is often increased in intensity due to recent fracture or surgery, healing osteoblastic activity or osteomyelitis. Other imaging methods are than necessary to differentiate between these possibilities for the increased uptake.

2. Is there an infection?

In patients with suspected PTO, with recent fracture and/or surgery, and osteosynthesis materials in situ, WBC scintigraphy is required. Increased uptake, increasing in size or intensity in time, indicates the presence of an infection.

In the late phase, and we do not know exactly the time point after fracture, probably 1-2 years, and without osteosynthesis in situ, FDG-PET is the best option, since this modality is easier to perform, has a high spatial resolution and only one imaging acquisition is necessary.

3. When there is an infection, where is it located: in the bone or in the soft tissues?
This question is easy to answer with the now existing hybrid camera systems. When available, always perform SPECT-CT when there is uptake visible at the WBC scintigraphy and always perform PET-CT when the FDG-PET modality is used. With the anatomical correlation it is easy to localize exactly the area of increased accumulation: in (osteomyelitis) or outside (soft tissue infection) the bone.

When should we use which nuclear technique?

In our opinion, these are the preferred nuclear imaging techniques for answering the different questions in a patient with suspected PTO (partly adapted from [17]):

- Non-union, is there vital bone: Three phase bone scan with SPECT-CT
- Non-union, is there an infection: WBC scintigraphy with SPECT-CT
- Suspected peripheral PTO, no surgery or surgery > 6 months ago and no osteosynthesis materials in situ: FDG-PET/CT
- Suspected peripheral PTO, osteosynthesis materials in situ, placement < 2 years ago: WBC scintigraphy with SPECT-CT
- Suspected peripheral PTO, osteosynthesis in situ, placement > 2 years ago: Three phase bone scan, followed by WBC scintigraphy with SPECT-CT if the bone scan is positive
- Suspected PTO located in the axial skeleton: FDG-PET/CT
- Suspicion for dissemination of infectious foci: FDG-PET/CT

EXAMPLES FROM CLINICAL PRACTICE

Patient A

Clinical story:

Patient A, a 37 year old healthy male, underwent open reduction and internal fixation (ORIF) of an open fracture of his right distal tibia and fibula 22 years ago. This was complicated by posttraumatic osteomyelitis and resulted in multiple re-operations with debridements of the bone, removal of most hardware and free flap coverage of a soft tissue defect. He was referred to our hospital with a persistent clinical infection around his right distal tibia and a near wound breakdown of the scar. Medical imaging was requested a) to confirm the diagnosis of osteomyelitis and b) to determine the anatomical location of the suspected osteomyelitis.

Imaging results:

First, according to the diagnostic imaging protocol in our hospital, a three phase bone scan was performed since the fracture and surgery was > 2 years ago. All three phases of the bone scan were positive, only the late phase (Figure 5, G: anterior view, H: lateral view) is showed. This increased osteoblastic uptake can be the result of an infection, but also due to a healing fracture or recent surgery. For further differentiation, the patient underwent a WBC scan (Figure 5, A-D: images after 4 hours, E-F: images after 24 hours). This showed increased uptake in intensity and size over time, suspect for an infection. To localize this accumulation of leukocytes a SPECT-CT was performed (Figure 6) which revealed that the uptake was located outside the bone, in the soft tissue. Final diagnosis was a soft tissue infection.

Patient B

Clinical story:

Patient B, a 46 year old schizophrenic but otherwise healthy male sustained an open and comminuted talar neck fracture after a fall from height. This was initially treated with multiple soft tissue debridements and an external fixation, later augmented with screw and K-wire fixation of the fracture. The soft tissue defect was closed with a local myocutaneous flap. Two months after this last procedure the patient presented with a draining sinus in the scar on the lateral side of the ankle joint. Medical imaging was requested to a) assess the viability of the talus and b) to determine the anatomic location of the suspected osteomyelitis. The X-ray is shown in Figure 7 (left image).

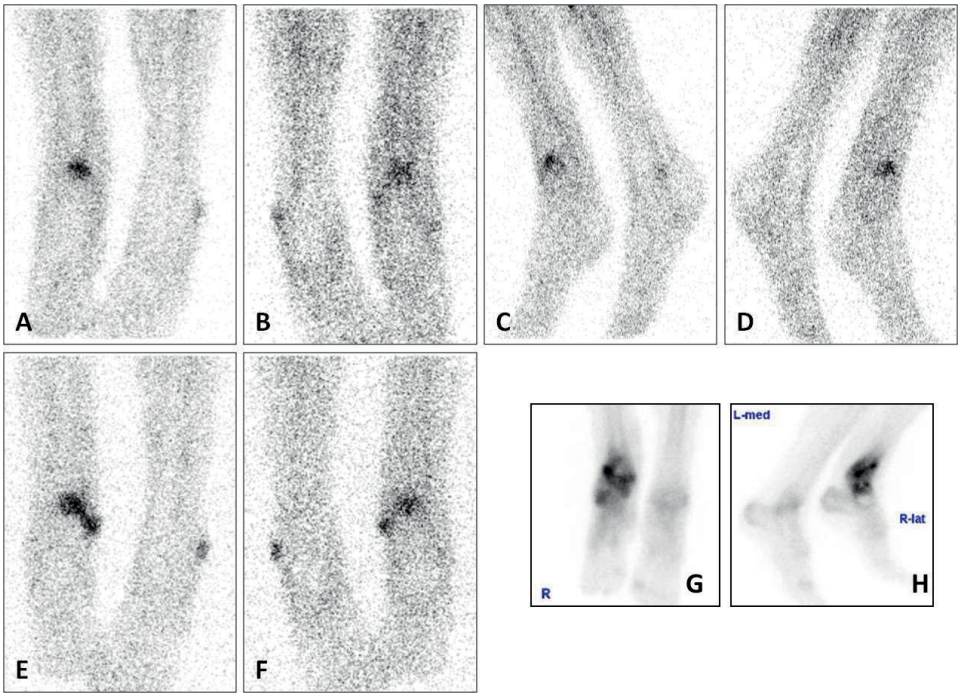

Figure 5. WBC scan (**A-D** images after 4 hours, **E-F** images after 24 hours) and late phase bone scan (**G** anterior view, **H** lateral view) of patient A

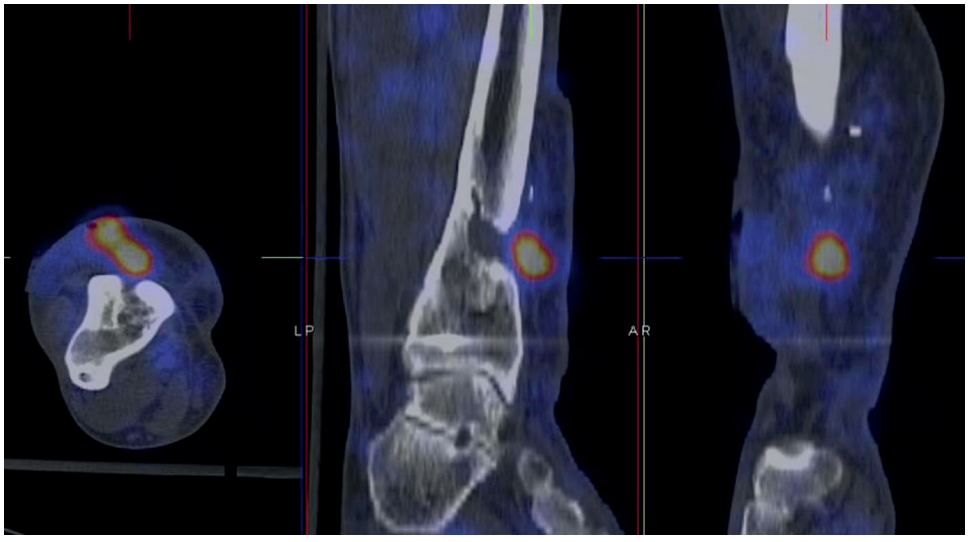

Figure 6. WBC scan SPECT-CT of patient A.

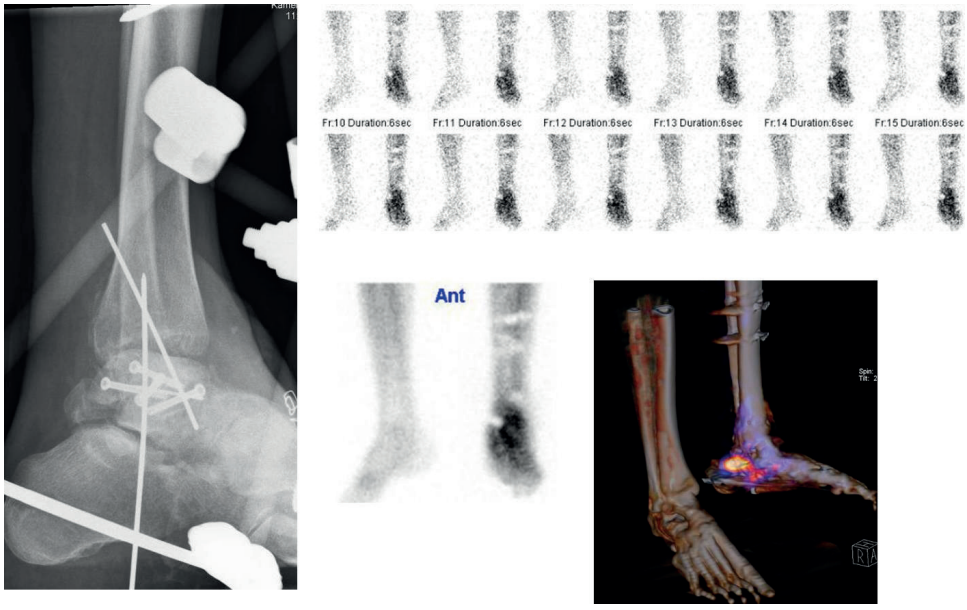

Figure 7. X-ray (left image) and bone scan (upper row: flow phase, middle image lower row: blood pool phase, right image lower row: SPECT-CT late phase) of patient B.

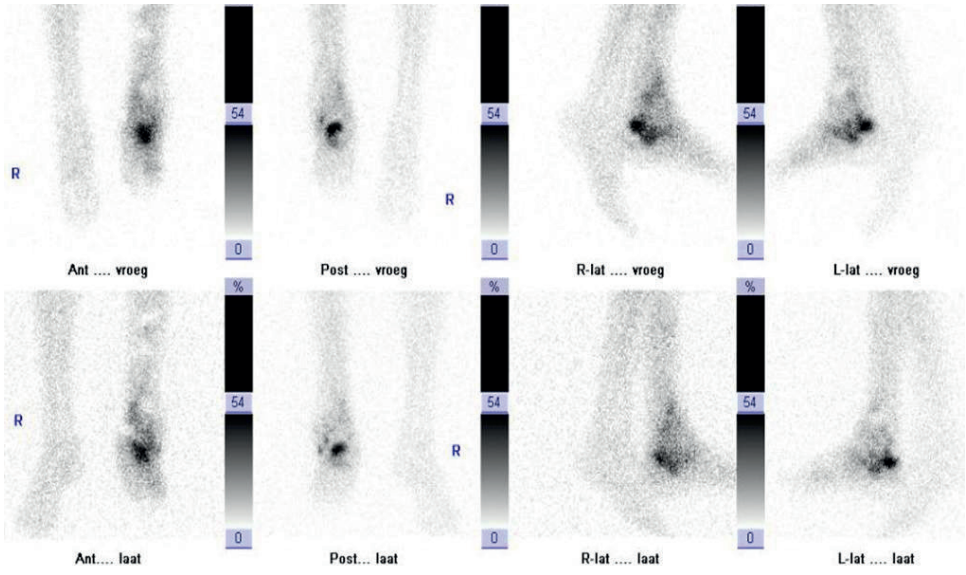

Figure 8. WBC scan (upper row: images after 4 hours, lower row: images after 24 hours) of patient B.

Imaging results:
To answer question a) a three phase bone scan was performed. The first phase (flow phase) is shown in Figure 7 (upper row images): positive flow at the talar region of the left foot. Obviously also the second phase (blood pool phase, middle image lower row) and the late phase (combined with CT image, right image lower row) are positive.

This means the bone is viable. However, differentiation between infection or healing fracture is not possible. Therefore, WBC scintigraphy was performed (Figure 8, upper row: images after 4 hours, lower row: images after 24 hours). The uptake decreases in time, meaning that the leukocyte accumulation is the result of a healing fracture and not of an osteomyelitis. After proper wound care further healing was uneventful.

Patient C

Clinical story:
Patient C, a 33 year old healthy male, was referred to our hospital because of a suspected posttraumatic osteomyelitis in combination with a malunion of his left tibia. He sustained a gunshot wound to his left lower leg in the middle east conflict two years prior to this presentation which was treated with a prolonged immobilization in an external fixator combined with several wound debridements. The last operation was only a few months prior to presentation. On examination, apart from the obvious malalignment of his left lower leg, we noted a closed but unstable scar on the medial side of his left tibia. Medical imaging was requested to a) confirm the diagnosis and b) to determine the anatomic location of the suspected osteomyelitis.

Imaging results:
Since his last surgery was < 6 months ago, immediately a WBC scan was performed (Figure 9, left image: anterior view after 4 hours, right image: anterior view after 24 hours): uptake is visible at 3 locations. When calculating the ratios (uptake focus – to – contralateral side) the uptake at the most proximal and most distal focus decreases in time. This means these uptakes are due to regeneration of bone marrow. However, the uptake at the middle focus increases in time, which is suspect for an infection. The SPECT-CT (Figure 10) shows the uptake in the bone and a small fistula to the bone marrow. Indeed, surgery revealed an infection at this location.

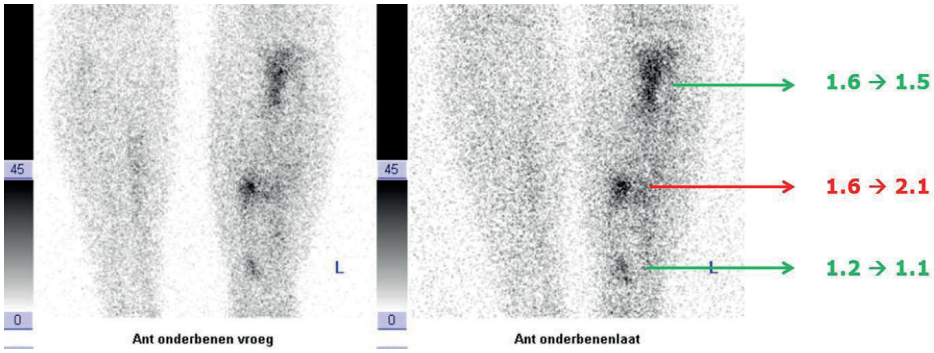

Figure 9. WBC scan (left image: anterior view after 4 hours, right image: anterior view after 24 hours) of patient C.

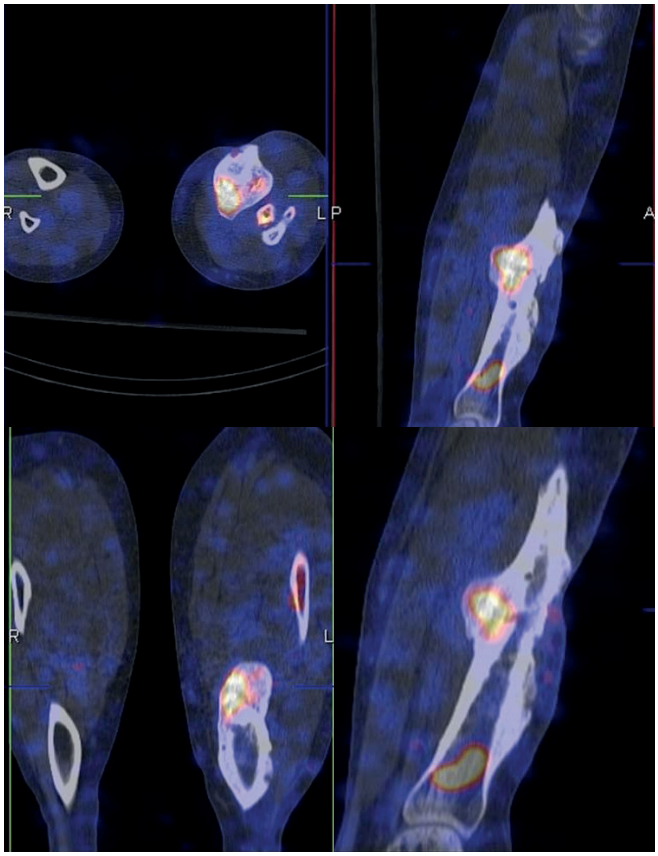

Figure 10. WBC SPECT-CT of patient C.

Table 1. Nuclear medicine modalities to image PTO

Imaging test	Major advantages	Major disadvantages	Relative costs (€)	Sensitivity/ specificity	When to order	Other comments
Bone scan + SPECT/CT	Widely available Cheap Negative bone scan excludes infection	Low specificity: increased uptake at all sites of increased bone metabolism irrespective of the underlying disease No role in acute PTO Probably positive for 1-2 years after ORIF	300-400	Sensitivity 80-90% Specificity 50-70%	Viable bone Suspected peripheral PTO, osteosynthesis in situ, placement > 2 years ago	Positive bone scan must be interpreted with caution and other imaging methods are necessary to differentiate between an infection and other causes of increased osteoblastic activity
WBC scan + SPECT/CT	Specific for leukocytic infiltration Accurately detects both acute and chronic infections High diagnostic accuracy	Laborious preparation Dual time point imaging necessary	800-1000	Sensitivity 80-100% Specificity 80-100%	Suspected infected non-union Suspected peripheral PTO, osteosynthesis materials in situ, placement < 2 years ago; or when bone scan is positive > 2 years	Correct acquisition, analysis and interpretation protocol has to be followed With SPECT-CT differentiation between osteomyelitis and soft tissue infection possible
FDG-PET/CT	Short acquisition time High image resolution No need for blood manipulation	Not possible to differentiate between infection and inflammation No existing criteria for positivity	1000-1200	Sensitivity 40-100%* Specificity 60-90%* *depending on which criteria for positivity are used	Suspected peripheral PTO, no surgery or surgery > 6 months ago and no osteosyn-thesis in situ Suspected PTO in the axial skeleton Suspicion for dissemination	Consensus criteria for positivity necessary

CONCLUSION

Nuclear medicine modalities play an important role in the assessment of posttraumatic osteomyelitis. Three phase bone scintigraphy can be used to exclude PTO in longstanding cases, but when positive other imaging techniques are necessary. WBC scintigraphy, when using the correct acquisition, analysis and interpretation protocols, is a specific technique to diagnose an infection with high diagnostic accuracy. FDG-PET has several advantages and can perfectly be used in a chronic non postoperative setting, but should be interpreted with caution when metal implants are in situ or when surgery was performed recently. The pros and cons of the three different techniques are depicted in Table 1.

Prospective studies comparing these nuclear medicine imaging techniques with radiological imaging techniques like MRI are necessary to provide evidence based diagnostic flowcharts in patients with suspected PTO.

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CHAPTER 4

Accuracy of diagnostic imaging modalities for peripheral posttraumatic osteomyelitis - a systematic review of the recent literature

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ABSTRACT

Aims. Post-traumatic osteomyelitis (PTO) is difficult to diagnose and there is no consensus on the best imaging strategy. The aim of this study is to present a systematic review of the recent literature on diagnostic imaging of PTO.

Methods. A literature search of the EMBASE and PubMed databases of the last 17 years (2000-2016) was performed. Studies that evaluated the accuracy of magnetic resonance imaging (MRI), three phase bone scintigraphy (TPBS), white blood cell (WBC) or antigranulocyte antibody (AGA) scintigraphy, fluorodeoxyglucose positron emission tomography (FDG-PET) and plain computed tomography (CT) in diagnosing PTO were considered for inclusion. The review was conducted using the PRISMA statement and QUADAS-2 criteria.

Results. The literature search identified 3,358 original records, of which 10 articles could be included in this review. Four of these studies had a comparative design which made it possible to report the results of, in total, 17 patient series. WBC (or AGA) scintigraphy and FDG-PET exhibit good accuracy for diagnosing PTO (sensitivity ranged from 50 -100%, specificity ranged from 40 -97% versus 83 - 100% and 51% - 100%, respectively). The accuracy of both modalities improved when a hybrid imaging technique (SPECT/CT & FDG-PET/CT) was performed. For FDG-PET/CT sensitivity ranged between 86 and 94% and specificity between 76 and 100%. For WBC-scintigraphy + SPECT/CT this is 100% and 89 - 97% respectively.

Conclusions. Based on the best available evidence of the last 16 years, both WBC (or AGA) scintigraphy combined with SPECT/CT or FDG-PET combined with CT have the best diagnostic accuracy for diagnosing peripheral PTO.

INTRODUCTION

Post-traumatic osteomyelitis (PTO), also known as "fracture-related" osteomyelitis, is a feared complication for its difficult recognition, significant treatment duration and high recurrence rate. Infection can present acutely in the first few weeks after internal fixation, in a delayed manner with low grade infection or late with infected non-union or persistent infection after fracture healing [1-3]. The incidence of deep infection after surgical fracture care is relatively high (between 1 and 19%), depending on trauma related risk factors such as contaminated open fractures, damage control procedures and concomitant soft tissue injuries [4-6]. Early treatment of acute infection can prevent progression to established PTO but this condition still affects 2-4% of all patients undergoing an open reduction and internal fixation of an open or closed fracture [7].

The key for a successful treatment of PTO is a prompt and accurate diagnosis. However, this diagnostic process in particular is challenging [7-19]. Many imaging modalities such as magnetic imaging resonance (MRI), three phase bone scintigraphy (TPBS), white blood cell (WBC) scintigraphy, antigranulocyte antibody (AGA) scintigraphy, fluorodeoxyglucose positron emission tomography (FDG-PET) and plain computed tomography (CT) are frequently used for diagnosing or excluding this condition. In the past 10 years there has been a huge development in new camera systems, combining nuclear medicine techniques such as Single Photon Emission Computed Tomography (SPECT) and PET with radiological techniques such as CT and MRI. Although these hybrid camera systems (SPECT-CT, PET-CT or PET-MRI) may lead to better localisation of the infection and as a consequence to better diagnostic accuracy rates, their diagnostic value for PTO has not yet been established [19-21].

The aim of this study is to present a systematic review of the recent literature (from 2000 to 2016) on imaging techniques to diagnose PTO.

MATERIALS AND METHOD

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [22] and its “Explanations and Elaboration” [23] were the guidance for this systematic review.

Search strategy

Following the recommendations of the Cochrane collaborations, a computerised literature search in the PubMed and Embase databases was conducted. Included were

articles in any language published between January 1st 2000 and December 31st 2016. Search terms (Table 1) were defined by two authors with the assistance of a professional information retrieval specialist. The Cochrane Library [24] was checked for reviews on diagnostic imaging modalities for osteomyelitis. In addition references of included studies and of relevant review articles, editorials and/or commentaries of the last 16 years were scrutinized for additional articles to be included.

Table 1. Search strings for Pubmed and Embase

PUBMED
("Osteomyelitis"[Mesh] OR "Osteitis"[Mesh] OR ("Surgical Wound Infection"[Mesh] AND bone*[tiab]) OR osteomyelitis[tiab] OR osteitis[tiab]) AND ("Diagnostic Imaging"[Mesh] OR "Magnetic Resonance Imaging"[Mesh] OR "Tomography, X-Ray"[Mesh] OR "Tomography, Emission-Computed"[Mesh] OR "Radionuclide Imaging"[Mesh] OR "Positron-Emission Tomography"[Mesh] OR "Fluorodeoxyglucose F18"[Mesh] OR "Leukocytes/radionuclide imaging"[Mesh] OR "Technetium Tc 99m Exametazime"[Mesh] OR diagnostic imaging[tiab] OR MRI[tiab] OR "bone scan"[tiab] OR "CT scan"[tiab] OR "computed tomography"[tiab] OR SPECT-CT[tiab] OR SPECT/CT[tiab] OR PET[tiab] OR PET/CT[tiab] OR PET-CT[tiab] OR FDG[tiab] OR fluorodeoxyglucose[tiab] OR scintigraphy[tiab]) NOT Case Reports[ptyp] AND PY: from 2000, added to Pubmed until dec2015
EMBASE
'osteomyelitis'/mj OR 'osteitis'/mj OR ('surgical infection'/exp/mj AND bone*:ab,ti) OR osteomyelitis:ab,ti OR osteitis:ab,ti AND ('diagnostic imaging'/exp OR 'nuclear magnetic resonance imaging'/exp OR 'tomography'/de OR 'computer assisted tomography'/exp OR 'emission tomography'/exp OR 'whole body tomography'/exp OR 'scintiscanning'/exp OR 'fluorodeoxyglucose f 18'/exp OR ('leukocyte'/exp/mj AND imaging) OR ('technetium 99m'/exp/mj AND imaging) OR 'diagnostic imaging':ab,ti OR mri:ab,ti OR 'bone scan':ab,ti OR 'ct scan':ab,ti OR 'computed tomography':ab,ti OR 'spect-ct':ab,ti OR 'spect/ct':ab,ti OR pet:ab,ti OR 'pet/ct':ab,ti OR 'pet-ct':ab,ti OR fdg:ab,ti OR fluorodeoxyglucose:ab,ti OR scintigraphy:ab,ti) NOT 'case report'/exp AND [2000-2016]/py AND [1-1-1900]/sd NOT [31-12-2015]/sd

Study selection

Emphasis in this review is on patients suffering from osteomyelitis of the peripheral skeleton that emerged after trauma-related injuries. Depending on the type of injury and previous treatment strategies, these could be implant-associated infections or not. For this reason articles reporting on diagnostic medical imaging techniques for other types of bone or non-trauma related infections were excluded. This review does not include cases of haematogenous osteomyelitis. The inclusion and exclusion criteria (Table 2) are in line with endpoints used in earlier meta-analyses on this topic [17, 25]. Only studies investigating widely available diagnostic imaging tests for osteomyelitis - which are TPBS, WBC (or AGA) scintigraphy, FDG-PET, MRI and CT-scan - were eligible for this review. This study is limited to PTO of the peripheral skeleton as the upper and lower limb are the most commonly affected anatomical regions. Furthermore some diagnostic nuclear imaging modalities have limitations in imaging the axial skeleton, as tracers

may behave differently and WBC-scintigraphy's are more difficult to interpret because high uptake of white blood cells in the liver, spleen and bone marrow may obscure the specific uptake [19, 26]. Therefore, osteomyelitis of the axial skeleton was not assessed in this review. No concessions were made for non-trauma related studies. Due to our desire to include the most relevant papers, we did allow a low number (<15%) of trauma related prosthetic joint infections (PJI) and non-peripheral PTO sites provided that this was clearly stated by the authors and the data could not be extricated otherwise. If applicable, this is mentioned explicitly in the results section of this paper. The procedure for inclusion of studies was based on the recommendations by Van Tulder et al. [27].

Table 2. Inclusion and exclusion criteria.

INCLUSION CRITERIA
1) The study must evaluate the accuracy of radiological and nuclear imaging modalities for diagnosing PTO.
2) The study group must be at least 10 patients of 18 years and older with (suspected) PTO. In case of a mixed population, the data for this subgroup must be available independently.
3) The studied location must be in the peripheral skeleton.
4) The study must use a valid reference test (osteomyelitis was proven histologically and/or bacteriologically, and/or there was a clinical follow-up of at least six months in which no signs or symptoms of chronic infection were described.
5) Studies must provide sufficient details to construct a 2x2 contingency table expressing the results of the index tests by the disease status.
6) The study must investigate a commonly used diagnostic imaging test for PTO. These are conventional X-ray, CT, MRI, WBC scintigraphy /AGA scintigraphy (+/- SPECT/CT), bone scintigraphy (+/- SPECT/CT) and FDG-PET (+/- CT).
EXCLUSION CRITERIA
1) Non-human studies.
2) Studies that investigate non-trauma related osteomyelitis (such as osteomyelitis due to spondylodiscitis, diabetic feet, haematogenous dissemination and pressure ulcers).
3) Studies that investigate a not commonly used diagnostic imaging test (such as ^{99m}Tc-ciprofloxacin (Infecton) scintigraphy or ^{68}Ga-citrate PET).

Methodological Quality Assessment

The qualitative assessment of study design was performed according to the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies, version 2) criteria as recommended by the Cochrane Institute. QUADAS-2 is a tool for the assessment of studies of diagnostic accuracy included in systematic reviews and consists of four domains: patient selection, index test, reference standard and flow and timing [28]. Each domain is assessed in terms of risk of bias, and the first three domains are also assessed in terms of concerns regarding the applicability of a study. Authors were contacted when information regarding the quality of the study was not provided in the articles.

Data extraction

The following data was extracted from all relevant papers: 1) author and journal; 2) year of publication; 3) type of study; 4) number of patients with PTO; 5) type of imaging modality; 6) gold standard; 7) data regarding diagnostic accuracy of the imaging modality for PTO; and 8) study limitations.

Statistical analysis

Data analysis was conducted in line with guidelines for systematic reviews from the Cochrane Collaboration. The discriminative ability of the imaging modalities was quantified by several measures of diagnostic accuracy: sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (PLR and NLR) and the diagnostic odds ratio (DOR), which were calculated based on raw data reported in the papers. NPV and PPV values range between 0 and 1, and high values can be interpreted as indicating the accuracy of the diagnostic test. The NLR is the ratio of the probability of a patient with PTO having a negative test result, and a patient without PTO having a negative test result. Similarly, the PLR is the ratio of the probability of a patient with PTO having a positive test result, and a patient without PTO having a positive test result. NLR values less than 1 indicate an increase in the probability of the absence of PTO. PLR values greater than 1 indicate an increase in the probability of PTO. The DOR of a test is the ratio of the odds of positive test results in persons with the disease relative to the odds of positive test results in the non-diseased. DOR ranges from zero to infinity, with higher values indicating better discriminatory test performance. When raw data were not available, the reported sensitivity and specificity measures were presented. A data analysis was conducted using Review Manager 5.3 (version 5.3.5, The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark).

Source of funding

No external funds were received in support of this study.

RESULTS

Included Studies

A total of 4,363 articles that met the initial search criteria were identified in PubMed (n=1,846) and Embase (n=2,517). The Cochrane Library contained 4 entries on imaging osteomyelitis, these were all meta-analyses of which two dealt with diabetic feet [29, 30], one with chronic, mostly posttraumatic osteomyelitis [17] and one with osteomyelitis of

unspecified aetiology [25]. Screening of the reference lists of these and other relevant articles found in PubMed [8, 9, 15, 18, 31-44] yielded 18 additional studies. After removal of duplicates (n= 1,023), 3,358 unique publications remained and were screened on title and abstract by two authors. This resulted in 141 titles, which were subsequently retrieved with the full text. The eligibility of each article was established by a group discussion until consensus was reached. One hundred and twenty-seven articles were excluded for specific reasons. Eventually, 14 studies remained for further analysis [21, 45-57] and underwent qualitative assessment according to the QUADAS-2 criteria by two authors (Table 3). This resulted in 4 more exclusions [50, 51, 56, 57]. For this process additional information was obtained by email from 2 corresponding authors [52, 55]. Finally, 10 studies [21, 45-49, 52-55] remained for inclusion in this systematic review. The inclusion process is summarized in Figure 1.

Table 3. QUADAS-2 assessment of applicability

Study	Risk of bias				Applicability concerns			Final decision
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard	Included/ Excluded
Ballani et al. 2007 [45]	?	?	?	?	☺	☺	☺	Included
Glaudemans et al. 2013 [47]	☺	☺	?	☺	☺	☺	☺	Included
Goebel et al. 2007 [46]	?	☺	☺	☺	☺	☺	☺	Included
Hartmann et al. 2007 [48]	?	☺	?	☺	☺	☺	☺	Included
Horger et al. 2003 [21]	?	☺	?	☺	☺	☺	☺	Included
Kaim et al. 2000 [49]	☹	☺	☺	☺	☺	☺	☺	Included
Ledermann et al. 2000 [50]	☹	☺	☺	☺	☹	☺	☺	Excluded
Mahnken et al. 2000 [51]	?	☺	?	?	☹	☺	?	Excluded
Meller et al. 2002 [52]	☺	☺	☺	?	☺	☺	☺	Included
Schiesser et al. 2003 [53]	☺	☺	☺	☺	☺	☺	☺	Included
Shemesh et al. 2015 [54]	?	?	☺	☺	☺	☺	☺	Included
Wenter et al. 2016 [55]	☺	☺	☺	☺	☺	☺	☺	Included
Winter de et al. 2001 [56]	☹	☺	☺	☺	☹	☺	☺	Excluded
Wolf et al. 2001 [57]	☺	?	☹	☹	☺	☹	☹	Excluded

☺ low risk; ☹ high risk; ? unclear risk

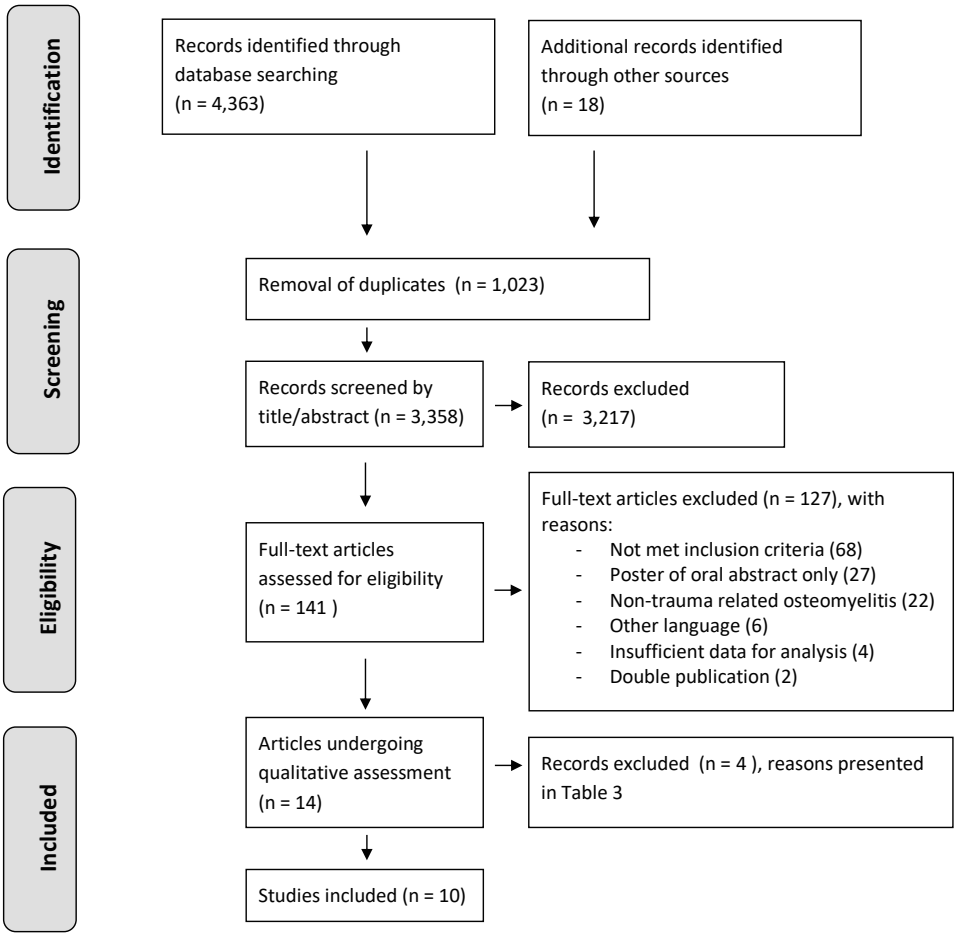

Figure 1. PRISMA Flow Diagram.

Study quality

Table 3 presents the final results of the risk of bias assessment. The risk of bias differed between studies. In general, there were concerns regarding patient selection and reference standards. The applicability of all studies was good.

Description of study characteristics

Four of the 10 articles [45, 46, 49, 52] had a comparative design which made it possible to include the results of in total 17 patient series (3 studies [46, 49, 52] investigated three imaging modalities). Six studies addressed the value of FDG-PET in the diagnostic

process for PTO [46, 48, 52-55], 5 studies WBC or AGA scintigraphy [21, 45, 47, 49, 52], 2 studies MRI [46, 49], 3 studies bone scintigraphy [45, 49, 52], and 1 study CT [46]. A schematic overview of the included studies is presented in Table 4. Due to the relatively small numbers of included studies and heterogeneity in applied diagnostic protocols, thresholds and cut-off points, pooling of data was not appropriate. Hence, results of individual studies are presented (Table 5).

Three phase bone scintigraphy

All three studies addressing the value of three phase bone scintigraphy for diagnosing PTO are comparative studies [45, 49, 52]. Ballani et al. [45] compared three phase ^{99m}Tc-methylene diphosphate (MDP) bone scintigraphy with ^{99m}Tc- hexamethylpropylene amine oxime (HMPAO) WBC-scintigraphy. They studied a total of 24 patients of whom 10 patients were suspected of suffering from PTO; all TPBS in this study were abnormal of which 4 were false positive. Kaim et al. [49] compared the value of combined TPBS / AGA scintigraphy with MRI for diagnosing PTO in a retrospective series with a highly selective patient group (19 suspected sites in 18 patients all with long-standing PTO). Meller et al. [52] performed TPBS as a selection tool for continuing with a WBC-scintigraphy (which was subsequently performed in 28 patients of whom 19 had 21 suspected sites of PTO). All 19 PTO patients had a positive result, of which only 4 were true positive.

Overall, the sensitivity of three phase bone scintigraphy was high (ranging from 89 to 100%), but the specificity was low (0 to 10%) (Table 5). The other accuracy measures showed that bone scintigraphy without additional imaging has low diagnostic value for detecting PTO.

WBC scintigraphy / AGA scintigraphy

The WBC scintigraphy and AGA scintigraphy studies are discussed together as both visualize the leukocyte infiltration within the patient. In WBC scintigraphy, the autologous white blood cells of patients are collected, labelled ex vivo and subsequently reinjected. In AGA scintigraphy commercially available labelled monoclonal antibodies against the granulocytes are directly injected and bind in the patient to the leucocytes. Five suitable studies [21, 45, 47, 49, 52] were identified addressing the value of WBC or AGA scintigraphy (2 studies combined with SPECT/CT [21, 47]) for diagnosing PTO. Ballani et al. [45] compared ^{99m}Tc-HMPAO WBC-scintigraphy with TPBS in a group of 24 patients with a clinical suspicion of osteomyelitis (of whom 10 with suspected PTO). A limitation of this study is that their acquisition protocol consisted of a rather high dose of injected ^{99m}Tc compared to current standards [47, 58] and they did not perform dual time point imaging (images 2-4 h and 20-24 h after reinjection).

Table 4. Schematic overview of included studies

Imaging modality	Author	Year	PTO Patients (n)	Specifics on imaging technique/ tracer	Use of hybrid imaging	Methodology	Timeframe between trauma, first symptoms of infection and diagnostic imaging
Three phase bone scintigraphy	Ballani et al [45]	2007	10	3-phase ^{99m}Tc-MDP bone scan, 740 MBq, γ-camera with 256x256 matrix (pixel size ~ 2 mm).	No	Retrospective. Gold standard: microbiology (n=5); otherwise overall clinical assessment, FU unknown	Unknown
	Kaim et al [49]	2000	18/19*	3-phase ^{99m}Tc-DPD bone scan, 740 MBq, γ-camera with 256x256 matrix.	No	Retrospective, highly selective patient group without orthopaedic implants or patients whose devices had been removed. Gold standard: microbiology (n=13); otherwise overall clinical assessment with minimum of 16 months FU	Longstanding PTO, time interval between last surgical intervention and present study was 6,5 years (3 months – 39 years). Time interval between last surgery and imaging not clear.
	Meller et al [52]	2002	19/21*	3-phase ^{99m}Tc-MDP bone scan, 740 MBq. 150,000 – 400,000 counts for each projection.	No	Prospective. Gold standard: MRI (n=19); histology/microbiology (n=12); FU 1-6 months	Symptoms of infection lasting for more than 6 weeks
WBC (or AGA) scintigraphy	Ballani et al [45]	2007	10	WBC scintigraphy with ^{99m}Tc-HMPAO labelled autologous WBCs, 740 MBq, γ-camera with a 256 x 256 matrix (pixel size ~ 2 mm). No late (24h) phase scan.	No	Retrospective. Gold standard: microbiology (n=5); otherwise overall clinical assessment	Unknown
	Glaudemans et al [47]	2013	49	WBC scintigraphy with ^{99m}Tc-HMPAO labelled autologous WBCs, 500 MBq. Late phase scan included.	Yes	Retrospective. Gold standard: microbiology (n = 13), otherwise overall clinical assessment at 6 months follow-up	Unknown
	Horger et al [21]	2003	27/29*	AGA scintigraphy with ^{99m}Tc labelled murine monoclonal antibodies. 750 MBq. Late phase scan included. γ-camera with 128x128 matrix.	Yes	Prospective. Gold standard: microbiology (n=18); otherwise overall clinical assessment with minimum of 6 months FU. 25 patients with suspected PTO (of which 1 non peripheral) and 2 (suspected) PJI	Reactivation of chronic PTO suspected because of clinical inflammatory symptoms or elevated laboratory markers. Timeframe not specified.
	Kaim et al [49]	2000	18/19*	AGA scintigraphy with ^{99m}Tc labelled murine IgG antibodies, 555 MBq, γ-camera with a matrix of 256x256. Only one imaging time point (17 h)	No	Retrospective, highly selective patient group without orthopaedic implants or patients whose devices had been removed. Gold standard: microbiology (n=13); otherwise overall clinical assessment with minimum of 16 months FU	Longstanding PTO, time interval between last surgical intervention and present study was 6,5 years (3 months – 39 years). Time interval between last surgery and imaging not clear.

	Meller et al [52]	2002	19/21*	WBC scintigraphy autologous labelled with ^{111}In, 18-37 MBq. Late phase scan included. 128x128 matrix, 250,000 – 500,000 counts for each projection.	No	Prospective. Gold standard: MRI (n=19); histology/microbiology (n=12); FU 1-6 months	Symptoms of infection lasting for more than 6 weeks
FDG-PET	Goebel et al [46]	2007	50	^{18}F-FDG PET, 200 MBq. 128x128 matrix.	No	Prospective. Gold standard: microbiology (n=50). 2 suspected trauma related PJI included.	Symptoms of infection lasting for more than 6 weeks
	Hartmann et al [48]	2006	23	^{18}F-FDG PET, 300-400 MBq.	Yes	Retrospective, 15 patients with osteosynthesis, 3 prosthesis, 5 no material in situ. All trauma related. Gold standard: microbiology (n=23)	Symptoms of infection lasting for more than 6 weeks or presence of recurrent osteomyelitis
	Meller et al [52]	2002	19/21*	^{18}F-FDG PET, 296 MBq	No	Prospective. Gold standard: MRI (n=19); histology/microbiology (n=12); FU 1-6 months	Symptoms of infection lasting for more than 6 weeks
	Schiesser et al [53]	2003	17/20*	^{18}F-FDG PET, 300-400 MBq	No	Prospective. Gold standard: microbiology (n=20), clinical FU 6 months	Pain at motion or rest for at least 6 weeks, interval between last surgical intervention and FDG-PET scan 6 weeks – 14 months
	Shemesh et al [54]	2015	10	^{18}F -FDG PET, 296-555 MBq.	Yes	Retrospective. Gold standard: microbiology (n=9, five cultures minimum); otherwise overall clinical assessment with minimum of 1 year FU	Time from initial surgery to PET/CT 2 months – 20 years
	Wenter et al [55]	2016	84 131	^{18}F-FDG PET, weight adapted dose (mean 252 +/- 76 MBq). 131 patients underwent PET/CT with mostly a full dose CT (n=130) with iv contrast (n=106).	No Yes	Retrospective. Gold standard: microbiology (n=143); otherwise overall clinical assessment with minimum of 1 year FU. 215 patients, 192 suspected PTO (of which 12 non peripheral), 11 (suspected) PJI	Causative event prior to PET scan dated back 12 ± 13 year in the clinically infected group and 10 ± 12 years in the clinically uninfected group.
MRI	Goebel et al [46]	2007	18	No details on MRI technique provided	n/a	Prospective. Gold standard: microbiology (n=18)	Symptoms of infection lasting for more than 6 weeks
	Kaim et al [49]	2000	18/19*	1.5-T MRI, slice thickness 3-10 mm. Both body coil (n=10) and extremity coil (n=8) used. All scans with iv gadolinium contrast.	n/a	Retrospective, highly selective patient group without orthopaedic implants or patients whose devices had been removed. Gold standard: microbiology (n=13); otherwise overall clinical assessment with minimum of 16 months FU	Longstanding PTO, time interval between last surgical intervention and present study was 6,5 years (3 months – 39 years). Time interval between last surgery and imaging not clear.
CT-scan	Goebel et al [46]	2007	22	No details on CT technique provided	n/a	Prospective. Gold standard: microbiology (n=22)	Symptoms of infection lasting for more than 6 weeks

* Presented as: number of patients/number of suspected peripheral PTO sites. Calculations are based on number of sites.
Abbreviations: MDP: Methylene Diphosphate, DPD: 3,3-diphosphono-1,2-propanedicarboxyl acid tetrasodium salt, HMPAO: Hexamethylpropylene amine oxime, FU: follow up, AGA: anti-granulocyte antibodies, SPECT: Single-photon emission computed tomography, CT: Computerized tomography, T: Tesla, EANM: European Association of Nuclear Medicine

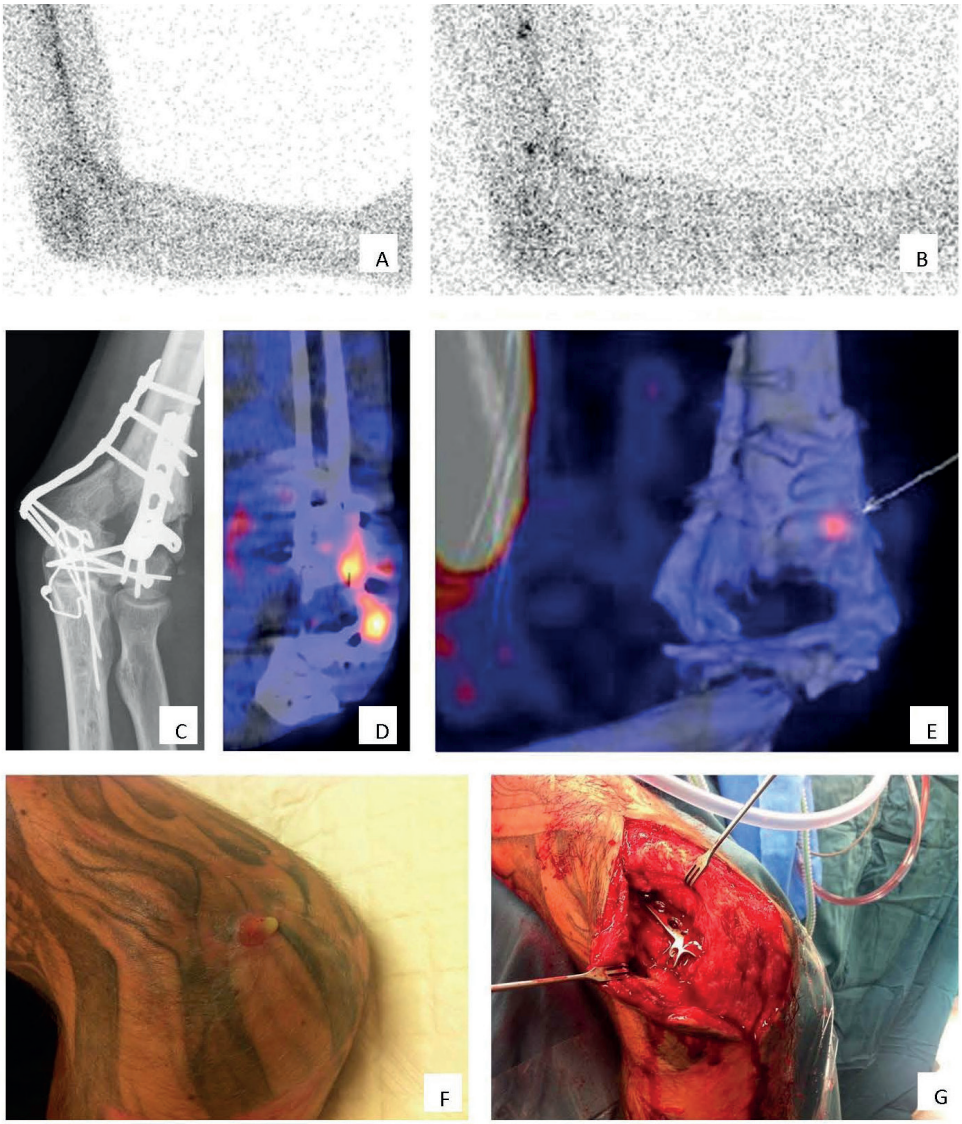

Figure 2. Clinical example WBC-scintigraphy + SPECT/CT. A 37 year old man with a grade 3A complicated distal humeral fracture of the left elbow, initially treated with an external fixator and subsequently by plate osteosynthesis of the distal humerus. **C** X-ray: situation after recent fixation of the fracture with plate ostheosynthesis, no signs of loosening or infection. After 4 months, he presented with a fistula and a clinical suspicion of osteomyelitis of the distal humerus. **A-B, D-E:** WBC scintigraphy (**A** image at 4 hours, **B** image at 24 hours, **D-E** fusion SPECT/CT images) after the injection of 220 MBq 99m-Tc-labeled leucocytes demonstrated an infection around the implant at the lateral side of the elbow/distal screw. The low uptake points to an only low grade appearance and the location to soft tissue involvement, this was confirmed at operation (**F** clinical pre-operative picture **G** perioperative clinical picture).

Glaudemans et al. [47] described the results of ^{99m}Tc-HMPAO WBC-scintigraphy in a large retrospective study with 297 patients with various musculoskeletal infections (of whom 49 patients had suspected PTO). Labelling protocols were in accordance with current EANM guidelines [58] and scans were acquired correctly with imaging at two time points. Diagnosis was confirmed by microbiology in 13 cases. Clinical follow-up of at least 6 months confirmed mainly negative cases in all other patients (additional information obtained from the author).

A prospective study by Horger et al. [21] of 27 patients undergoing scintigraphy with technetium-99m labelled AGA combined with SPECT/CT in 25 patients for 27 suspected PTO sites (including one non-peripheral location) and 2 suspected PJI is reported. This focused specifically on the added value of CT with SPECT. Sensitivity was identical for WBC-scintigraphy with SPECT alone and combined SPECT/CT (both 100%), whereas adding CT to SPECT improved the specificity from 78% to 89%. Kaim et al. [49] in a previously mentioned retrospective study compared the validity of combined TPBS/ ^{99m}Tc labelled AGA scan with MRI for diagnosing PTO (18 patients, 19 infected peripheral sites). In this paper the accuracy of the nuclear imaging was presented as a combined value for the TPBS and the AGA scan together. Again, imaging was only performed at one imaging time point (17 h after injection), which is a major limitation of this study. Finally, Meller et al. [52] reported on a comparative prospective study (^{111}InWBC-scintigraphy versus FDG-PET) with 30 consecutive chronic osteomyelitis patients of whom 19 PTO patients with 21 suspected infected sites in the peripheral skeleton.

Overall, sensitivity of WBC and AGA scintigraphy ranged from 50 to 100%, specificity ranged from 40 to 97% (Table 5). LR+ ranged from 1.30 to 33.33 and LR- values of 0.56 and 0.57 were found. These results indicate strong to convincing diagnostic evidence of WBC and AGA scintigraphy to accurately detect, and weak evidence to exclude, PTO. However, one should bear in mind that the labelling procedures, acquisition protocols and interpretation criteria of the WBC/AGA scintigraphy might be different between some 'dedicated' centres, which can have some impact on the results. DOR values of 2.32 and 7.46 were calculated, showing that the odds of obtaining a positive test results was 2.32 to 7.46 times higher in a person with PTO than in a person without PTO. Additionally, the studies that used SPECT/CT in combination with WBC (or AGA) scintigraphy reported higher diagnostic accuracy.

FDG-PET(/CT)

Six studies [46, 48, 52-55] were included addressing the value of FDG-PET in diagnosing PTO, 3 combined with CT [48, 54, 55]. Goebel et al. [46] prospectively investigated the diagnostic value of FDG-PET in 48 patients with peripheral PTO and compared this with

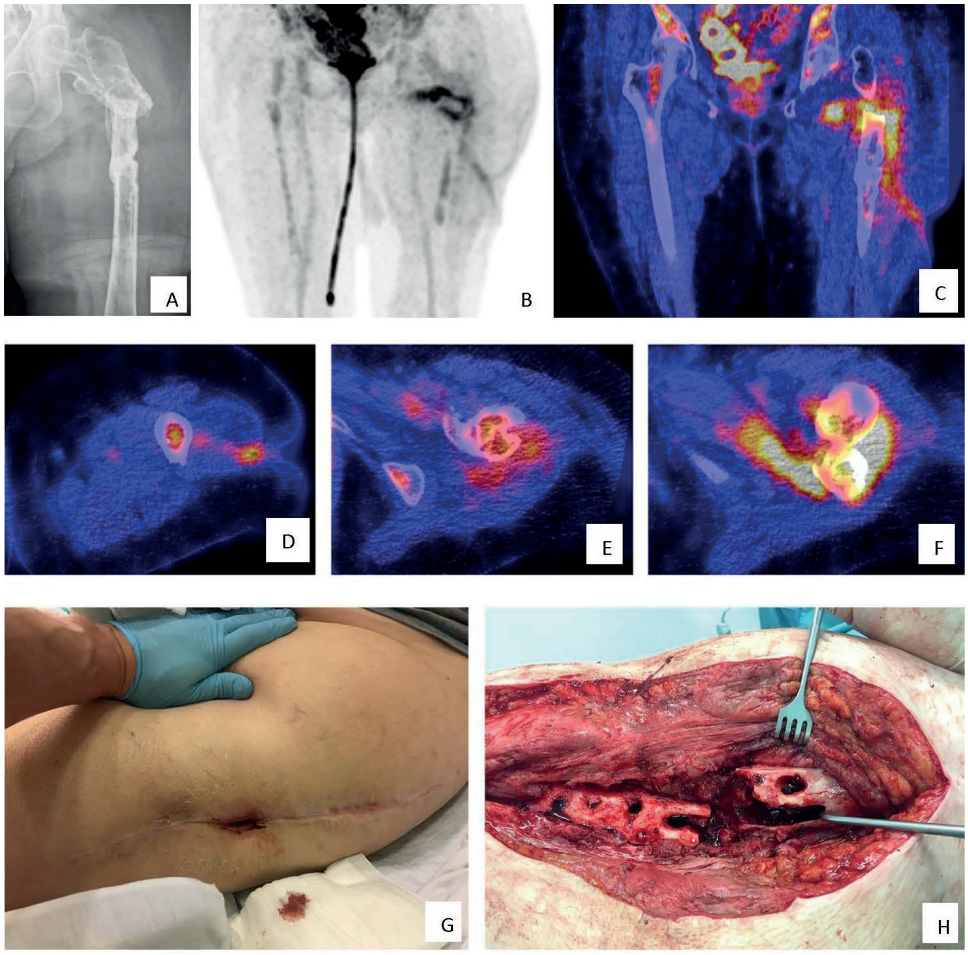

Figure 3. Clinical example FDG-PET/CT. A 77 year old woman who had a proximal femur fracture for which she underwent open reduction and internal fixation with a femur plate which had to be removed at a later stage due to infection. **A** X-ray, AP view: no consolidation, severe angulation, heterogeneous sclerotic aspect around the fracture. She was referred to our hospital with a fistula in the lateral thigh and a clinical suspicion of osteomyelitis of the proximal femur. Further imaging demonstrated an infection of the proximal femur, a medial abscess and a fistula coursing to the lateral aspect of the thigh which correlated with the clinical findings during surgery. **B-F** ^{18}F FDG PET/CT (**B** coronal FDG-PET image, **C** coronal fused FDG-PET/CT image, **D-F** transaxial fused FDG-PET/CT images). **G** clinical pre-operative picture **H** perioperative clinical picture.

CT (n=22) and MRI (n=18). Hartmann et al. [48] prospectively investigated 33 patients with FDG-PET/CT for suspected PTO, of which 23 had suspected PTO of the peripheral skeleton. Three patients in this study had a (suspected) trauma related PJI. Meller et al. [52] prospectively compared FDG-PET with ^{111}In-WBC in 30 consecutive patients (of whom 19 suspected of having peripheral PTO in 21 limbs), by using a dual-head coincidence camera. Schiesser et al. [53] prospectively analysed 17 patients with 20 suspected peripheral PTO sites using FDG-PET. Shemesh et al. [54] retrospectively looked at implant-related infections of the tibia in 10 patients investigated with FDG-PET/CT. Wenter et al. [55] reported the largest and most recent series of patients with PTO. They retrospectively reviewed the contributions of FDG-PET (n=84) and FDG-PET/CT (n=131) in a total of 215 patients with suspected PTO. If combined with CT, this was performed in the majority of patients with a full dose CT (n=130) and with IV contrast (n=106). The inclusion period was between 2000 and 2013; none of the patients had obvious signs of infection, 12 patients had suspected PJI and 12 non-peripheral suspected PTO sites were included.

Overall, sensitivity ranged from 83 to 100%, and specificity ranged from 51 to 100% (Table 5). The other measures showed moderate to strong diagnostic evidence of FDG-PET for either detecting or excluding PTO. Moreover, when the FDG-PET was combined with PET/CT the diagnostic accuracy measures increased significantly.

MRI

Two studies were included addressing the value of MRI in diagnosing PTO [46, 49], both with a comparative design. In the study of Goebel et al. [46], MRI (Tesla strength not reported) was performed in 18 of 50 patients with suspected PTO. Kaim et al. [49] carried out a retrospective study comparing the value of a combined TPBS / AGA scan with a 1.5 Tesla MRI for diagnosing PTO in a highly selective patient group (19 suspected sites in 18 patients all with long-standing PTO). All patients had T1 weighted images, 6/18 had T2 weighted images with fat suppression and 12/18 had T2 weighted images without fat suppression. All 18 had gadolinium enhancement. The third included study that describes the results of MRI for imaging PTO is the study by Meller et al [52]. Unfortunately this study could not be included in this review for the results of the MRI because only 7 patients with PTO of the peripheral skeleton underwent an MRI. Also, the authors used MRI as an adjudicator when no histology was available, therefore sensitivity and specificity of the MRI for PTO was not evaluated in this paper and could not be calculated from the data given.

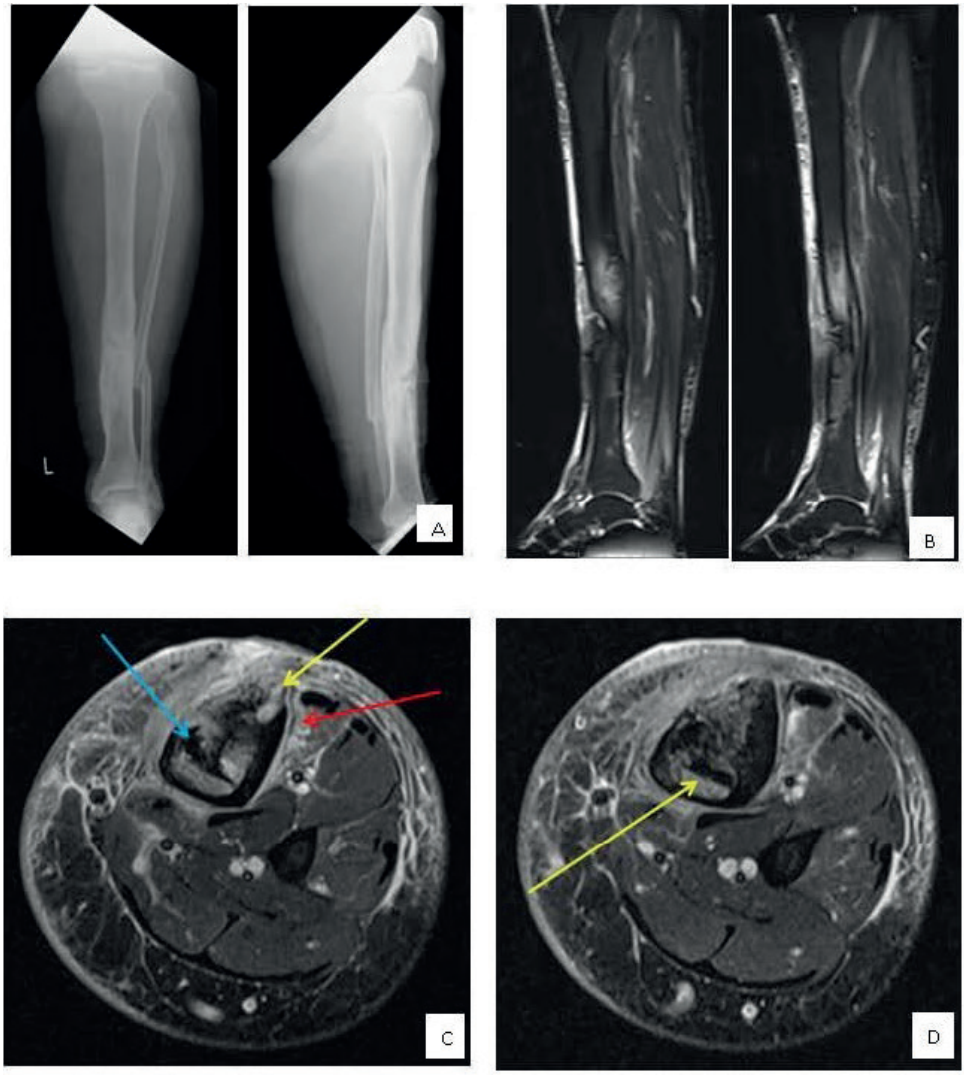

Figure 4. Clinical example MRI. A 54 year old man with a history of an open fracture treated with a plate many years ago. The fracture healed slowly and then the plate was removed because of continued skin breakdown over the front of the tibia. **A** Frontal and lateral radiograph demonstrating sclerosis and chronic periosteal reaction around the previous fracture site. **B** Sagittal fat suppressed images of the calf demonstrating bone and soft tissue oedema **C & D** Axial fat suppressed images demonstrating sequestra (blue arrow), cortical abscesses (yellow arrows) and periostitis and soft tissue oedema (red arrow).

Table 5. Diagnostic accuracy measures

Imaging modality	Author	Use of Hybrid imaging	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	LR+	LR-	DOR
Bone scintigraphy	Ballani et al. [45]	No	1.00 (0.54, 1.00)	0.00 (0.00, 0.60)	0.60	NE	NE	NE	NE
	Kaim et al. [49]	No	0.89 (0.52, 1.00)	0.10 (0.00, 0.45)	0.47	0.50	0.99	1.11	0.89
	Meller et al. [52]	No	1.00 (0.40, 1.00)	0.00 (0.00, 0.20)	0.19	NE	NE	NE	NE
WBC (or AGA) scintigraphy	Ballani et al. [45]	No	1.00 (0.59, 1.00)	0.67 (0.09, 0.99)	0.88	NE	3.03	NE	NE
	Glaudemans et al. [47]	Yes	1.00	0.97	NE	NE	33.33	NE	NE
	Horger et al. [21]	Yes	1.00 (0.83, 1.00)	0.89 (0.52, 1.00)	0.95	NE	9.09	NE	NE
	Kaim et al. [49]	No	0.78 (0.40, 0.97)	0.40 (0.12, 0.74)	0.54	0.67	1.30	0.56	2.32
	Meller et al. [52]	No	0.50 (0.07, 0.93)	0.88 (0.64, 0.99)	0.50	0.88	4.25	0.57	7.46
FDG-PET	Goebel et al. [46]	No	0.92 (0.78, 0.98)	0.69 (0.39, 0.91)	0.89	0.75	3.00	0.12	25.00
	Hartmann et al. [48]	Yes	0.94 (0.73, 1.00)	0.87 (0.60, 0.98)	0.89	0.93	7.08	0.06	118.00
	Meller et al. [52]	No	1.00 (0.40, 1.00)	0.88 (0.64, 0.99)	0.67	NE	8.33	NE	NE
	Schiesser et al. [53]	No	1.00 (0.74, 1.00)	0.88 (0.47, 1.00)	0.92	NE	8.33	NE	NE
	Shemesh et al. [54]	Yes	0.86 (0.42, 1.00)	1.00 (0.29, 1.00)	NE	0.75	NE	0.14	NE
	Wenter et al. [55]	No	0.83	0.51	NE	NE	1.69	0.33	5.12
	Wenter et al. [55]	Yes	0.88	0.76	NE	NE	3.67	0.16	22.94
MRI	Goebel et al. [46]	n.a.	0.82 (0.48, 0.98)	0.43 (0.10, 0.82)	0.69	0.60	1.43	0.42	3.40
	Kaim et al. [49]	n.a.	1.00 (0.66, 1.00)	0.60 (0.26, 0.82)	0.69	NE	2.50	NE	NE
CT-scan	Goebel et al. [46]	n.a.	0.47 (0.23, 0.72)	0.60 (0.15, 0.95)	0.80	0.25	1.18	0.88	1.34

Abbreviations: PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, DOR: diagnostic odds ratio, NE: not estimable, n.a.: not applicable. The studies utilizing SPECT/CT or PET/CT are marked in blue.

Overall, sensitivity values of 82 and 100% and specificity values of 43% and 60% were found in the studies of Goebel et al. [46] and Kaim et al. [49], respectively (Table 5). The other measures showed weak evidence of MRI for diagnosing or excluding PTO.

CT

Only 1 study addressed the value of CT-scanning in diagnosing PTO (Goebel et al. [46]). Unfortunately, the technical aspects (number of slices and slice thickness) of the CT-scan used in this study are not reported. For the 22 patients with suspected PTO who were analysed with CT, they found a sensitivity of 47% and a specificity of 60% (Table 5). The other measures showed weak diagnostic evidence of CT for diagnosing or excluding PTO.

DISCUSSION

Based on the best available evidence over the last 16 years, as presented in this paper, both WBC (or AGA) scintigraphy and FDG-PET have the best diagnostic accuracy for diagnosing or excluding peripheral PTO. The sensitivity for WBC (or AGA) scintigraphy ranged from 50 to 100%, specificity ranged from 40 to 97%. For FDG-PET this was 83 to 100% and 51% to 100%, respectively. Moreover, the studies, which combined the WBC/AGA scintigraphy with SPECT/CT [21, 47] or the FDG-PET with PET-CT [48, 54, 55] (which is in line with current practice) showed an increase in the diagnostic accuracy measures. For FDG-PET/CT sensitivity ranged between 86 and 94% and specificity between 76 and 100%. For WBC-scintigraphy + SPECT/CT this is 100% and 89 - 97% respectively. These results do partly concur with the previous reported accuracy on diagnostic imaging of chronic osteomyelitis by Termaat et al. [17]. They included in their meta-analysis papers published between 1975 and 2003 and favoured FDG-PET as the optimal imaging modality. However, studies included for FDG-PET consisted mainly of patients suspected of chronic osteomyelitis and not specifically PTO. Furthermore, in that era, almost no SPECT/CT or PET/CT camera systems existed and acquisition protocols especially for WBC-scintigraphy have significantly improved since then [47, 58]. Glaudemans et al [47] presented the results of a more recent large retrospective study including 297 patients with suspected bone or soft tissue infection of whom 49 PTO patients analysed by WBC-scintigraphy. Fourteen of the 49 PTO patients had a positive scan result and were therefore further analysed with SPECT/CT. For PTO they found a sensitivity of 100%, a specificity of 97.4% and a diagnostic accuracy of 98%. Important to mention is that in this study labelling protocols were in accordance with current EANM guidelines [58] and scans were acquired correctly with imaging at two time points which make these results more in accordance with current practice.

Choosing the most appropriate imaging technique for PTO remains difficult because there are advantages, disadvantages, pitfalls and contraindications of each option within the field of both nuclear medicine and clinical radiology. First of all, PTO is a condition that occurs in a very heterogeneous patient population. Limited mobility of the patient might not allow dual time point imaging and location of the infection, co-morbidities and metal implants may affect the accuracy of the imaging techniques used. Secondly, what the surgeon needs to establish for proper pre-operative planning is not only the presence of an infection, but also whether there are specific features such as sequestra, cloacae, sinus tracts and intracortical or soft tissue abscesses present. This is also important in cases with no doubt about the diagnosis (for example in patients with fistula or exposed metalwork) where imaging methods can be used with lower specificity and sensitivity for detecting PTO (such as a MRI scan). Thirdly, for pre-operative planning it is important to determine fracture position, fracture union and to assess the integrity of implants. This is usually done by more conventional imaging methods which can sometimes be incorporated in the diagnostic workup of PTO (for example: a CT scan to assess fracture union can be omitted when a WBC-scintigraphy with SPECT/CT is performed). All these factors need to be taken into account when ordering or advising a specific imaging technique. Establishing the diagnosis of infection is the first requirement for investigating PTO but, as mentioned before, imaging must also give information which allows planning of effective surgical treatment by defining the anatomical distribution of the infected or dead bone. The specific advantages and disadvantages of each imaging modality are summarized below.

Bone scintigraphy alone is not suitable for diagnosing PTO because of its low specificity but it is relatively cheap and easy to perform with a high sensitivity. Therefore in chronic cases with low suspicion of PTO a normal bone scan can be used to exclude an infection.

WBC (or AGA) scintigraphy is a useful technique to diagnose PTO because leucocytes actively migrate to the site of infection and are therefore a more specific indicator for osteomyelitis. Also, the addition of the SPECT-CT allows better anatomical localisation and distinction between bone and soft tissue infections. A disadvantage is that performing a WBC-scintigraphy is expensive, laborious and time consuming (with strict labelling protocols and at least 2 scans at two following days [19, 47, 58]).

FDG-PET is a relatively quicker whole body imaging procedure (one imaging time point 60 minutes after injection) that can be used to detect multiple foci throughout the body. Disadvantages are that recent fractures and the presence of metallic hardware may decrease the accuracy of FDG-PET since FDG uptake will also be increased in inflammatory reactions [59]. Better spatial resolution and metal artefact reduction

techniques have improved the quality of both MRI and CT over the last decade [60, 61] and the low costs, quick scanning time and availability make these scans an attractive first choice for many surgeons.

Plain X-Rays and CT are specifically useful to image the degree of fracture union and to search for small sequestra but are less suitable for determining the exact localisation of infected bone.

MRI can demonstrate the extent of bone and soft tissue involvement in cases of PTO but an absolute requirement is that both the surgeon and imaging specialist need to be experienced with interpreting the images in order not to be distracted by physiological changes (such as bone oedema) or accompanying normal tissue healing. The increasing use of internal fixation of fractures makes MRI less useful in the early diagnosis of PTO.

Clinical examples of the use of WBC-scintigraphy + SPECT/CT, FDG-PET/CT and MRI for the surgical workup of patients with PTO are presented in Figure 2, Figure 3 and Figure 4 respectively.

Clinicians need to be aware of the advantages and limitations of each imaging modality and the potential diagnostic accuracy. The issues of patient comfort, safety and personal experience of the surgeon and imaging specialist are of importance in choosing appropriate imaging techniques [12, 19, 59]. This review highlights the fact that the evidence in the literature is still limited and hampered by heterogeneous patient populations and quickly evolving imaging techniques. It is therefore clear that there is a need for further prospective studies on diagnostic imaging of PTO.

Limitations of this study

Firstly, this study provides level 3 evidence on diagnostic imaging of PTO. The number of studies that could be included is limited, imaging techniques are heterogeneous and only four prospective studies met the inclusion criteria. Secondly, the studies were aimed at diagnosing or excluding PTO and did not focus on determining the anatomic distribution of infection for surgical planning. Thirdly, the studies provided limited information on the combination of hybrid imaging techniques such as SPECT/CT and PET/CT for detecting PTO and its extent.

CONCLUSION

Based on the best available evidence of the last 17 years, both WBC (or AGA) scintigraphy combined with SPECT/CT or FDG-PET combined with CT have the best diagnostic accuracy for diagnosing peripheral PTO.

Compliance with ethical standards

Ethical approval: This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest

None

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CHAPTER 5

High diagnostic accuracy of white blood cell scintigraphy for fracture-related infections: results of a large retrospective single-center study

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ABSTRACT

Introduction. White blood cell (WBC) scintigraphy for diagnosing fracture-related infections (FRIs) has only been investigated in small patient series. Aims of this study were (1) to establish the accuracy of WBC scintigraphy for diagnosing FRIs, and (2) to investigate whether the duration of the time interval between surgery and WBC scintigraphy influences its accuracy.

Patients and methods. 192 consecutive WBC scintigraphies with ^{99m}Tc-HMPAO-labelled autologous leucocytes performed for suspected peripheral FRI were included. The golden standard was based on the outcome of microbiological investigation in case of surgery, or – when these were not available – on clinical follow-up of at least six months. The discriminative ability of the imaging modalities was quantified by several measures of diagnostic accuracy. A multivariable logistic regression analysis was performed to identify predictive variables of a false-positive or false-negative WBC scintigraphy test result.

Results. WBC scintigraphy had a sensitivity of 0.79, a specificity of 0.97, a positive predicting value of 0.91, a negative predicting value of 0.93 and a diagnostic accuracy of 0.92 for detecting an FRI in the peripheral skeleton. The duration of the interval between surgery and the WBC scintigraphy did not influence its diagnostic accuracy; neither did concomitant use of antibiotics or NSAIDs. There were 11 patients with a false-negative (FN) WBC scintigraphy, the majority of these patients (n=9, 82%) suffered from an infected nonunion. Four patients had a false-positive (FP) WBC scintigraphy.

Conclusions. WBC scintigraphy showed a high diagnostic accuracy (0.92) for detecting FRIs in the peripheral skeleton. Duration of the time interval between surgery for the initial injury and the WBC did not influence the results which indicate that WBC scintigraphy is accurate shortly after surgery.

INTRODUCTION

Postoperative infection is one of the most common and yet also most severe complications associated with the surgical fixation of fractures. It results in increased morbidity, higher medical costs and prolonged hospital admission times [1, 2]. Despite new and promising methods to prevent and treat these infections, the key to successful management is to establish an early and correct diagnosis. A distinction used to be made between early and late infections. However, classifications are rather arbitrary and there is no scientific evidence that the timing of the onset of the infection has any effect on the diagnostic tests or the treatment outcome. As a result, new definitions are being developed. In a recent consensus meeting supported by the Association for the Study of Internal Fixation (*Arbeitsgemeinschaft für Osteosynthesefragen*, AO) it was agreed to refer to the complete spectrum of infections following surgical fixation of a closed or open fracture as "fracture-related infection" (FRI) when no further detailed information is available on the degree of bone involvement and clear definition criteria now exist [3]. Many factors (both patient-related and doctor- or health institution-related) play a role in the clinical decision-making process for diagnosing FRI [4, 5]. Usually it is not early FRIs which pose a diagnostic challenge. Clinical signs of early infections are clear and tend to be easily recognizable, requiring limited or no additional diagnostic imaging. From a diagnostic perspective it is the established form of FRI which can be difficult to diagnose, and advanced medical imaging can be indispensable. However, most studies that investigate the accuracy of these advanced imaging modalities are aimed at hematogenous osteomyelitis and prosthetic joint infections (PJIs), or are conducted in an inhomogeneous orthopedic population with infections of different etiology [6-8]. FRIs are a different entity than PJIs because fracture fixation implants are in situ, which often allows micro-motion of the surrounding bone in contrast to an articulating but otherwise rigid prosthesis. There may also be confounding factors present such as a recent fracture with regenerating bone, soft-tissue disruption and/or other concomitant injuries. In many countries white blood cell (WBC) scintigraphy is a commonly utilized imaging modality for orthopedic infections, including FRI [9, 10]. However, although labeling and interpretation guidelines for WBC scintigraphy are now set by the European Association of Nuclear Medicine (EANM) [11], evidence of its accuracy in FRIs is limited in the literature [12]. There is also uncertainty regarding the minimum time interval required between surgery and WBC scintigraphy in order to minimize postoperative artifacts (and therefore false-positive results). As a FRI is a surgical complication it is important to know how previous surgery influences the outcome of any diagnostic test.

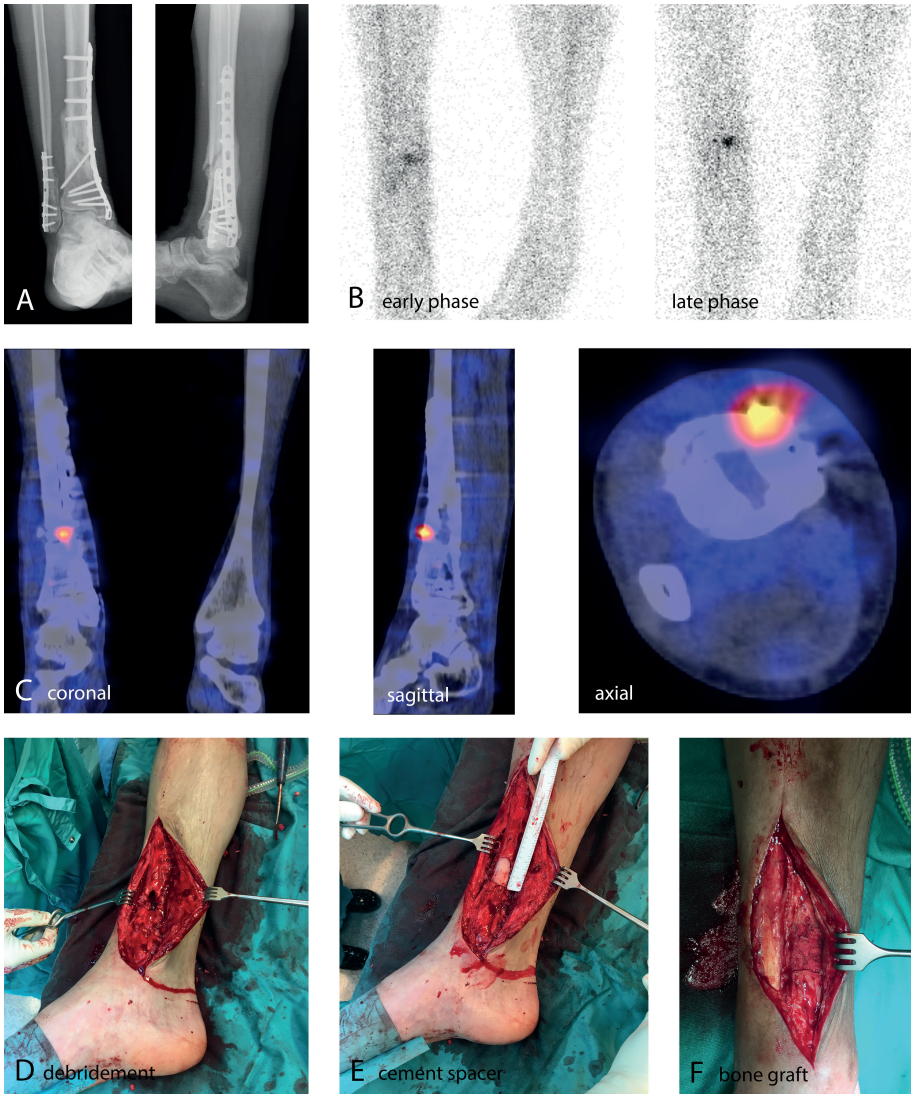

Figure 1. Clinical example. A 57-year-old man was treated with a plate osteosynthesis for a crural fracture **(A)** 10 months ago. He presented with a small area of redness and tenderness at the distal-medial tibia. WBC scintigraphy showed an increased uptake in the distal tibia in both the early and late phases **(B)** of the scan, with intensity increasing in time. A SPECT/CT **(C)** demonstrated a hotspot around the lag screw in the tibia. The patient was operated and there was an infection detected around the lag screw. All osteosynthetic materials were removed. Subsequently, the infected bone around the lag screw was debrided **(D)** and a cement spacer **(E)** was temporarily placed in a small tibial bone defect. Cultures of the implants demonstrated an ochrobactrum intermedium microorganism. After IV and oral treatment with antibiotics, the bone defect was filled with a cancellous bone graft during the second stage of this Masquelet procedure **(F)**. The patient recovered uneventfully.

To resolve these questions, the two aims of this study were:

1) to establish the accuracy of WBC scintigraphy for diagnosing FRI in a large and homogeneous patient group.
2) to investigate whether the duration of the time interval between surgery and WBC scintigraphy influences its diagnostic accuracy.

Patients and methods

The local hospital information system was reviewed for all consecutive patients who underwent a WBC scintigraphy for suspected FRI between 1 February 2009 and 8 November 2016. FRI was defined as any bone infection resulting from the surgical or non-surgical treatment of an open or closed fracture. The index event was the date of injury for an open conservatively managed fracture and the date of the operation in case of surgery. The WBC scintigraphy had to be aimed at diagnosing or excluding an FRI in relation to the index event. Patients with other types of orthopedic infections (such as PJI, spondylodiscitis and hematogenous osteomyelitis) were excluded. Additionally, the suspected FRI had to be located in the peripheral skeleton, as 1) the upper and lower limb are the most commonly affected anatomical regions and 2) WBC scintigraphy does have limitations in imaging the axial skeleton due to the relatively high uptake of white blood cells in the liver, spleen and bone marrow, and as such may obscure the specific uptake [5, 12, 13].

The final diagnosis of FRI (the gold standard) was based on the outcome of medical microbiological (MMB) investigations in case of surgery, or – when these were not available – on clinical follow-up of at least six months. Judgment of validity of the MMB results was done by an experienced trauma surgeon. The microbiological results of swabs and cultures of fistulas were disregarded due to relatively low accuracy [14-16]. Only if more than two deep-tissue cultures were available from the suspected site were the MMB results accepted as relevant. If this was not the case, the final diagnosis was based on clinical follow-up. A positive MMB was defined as accurate microbiology sampling combined with at least two positive cultures with the same organism [17, 18]. A positive result at clinical follow-up was defined as any wound break down, the presence or development of a sinus tract or redness and/or swelling that prompted the clinician to start the patient on antibiotic treatment or commence surgery. If the patient had an operation because of a suspected infection due to clinical symptoms after an initially negative WBC-scan the MMB results were evaluated and taken into account in regard to the initial scan.

The electronic patient files of all included patients were retrospectively reviewed to collect all data, including demographic details, information on the use of steroids and/or antibiotics, mechanism of injury, type of fracture according to the Müller AO Classification of Fractures [19], Gustilo Anderson classification in case of an open fracture [20], microbiological results, and clinical details at follow-up. The anesthesia files were reviewed for the American Society of Anesthesiologists (ASA) classification [21], Body Mass Index (BMI), comorbidities, use of nicotine and/or recreational drugs, and use of other relevant medication.

Ethical approval

Due to the observational nature of this analysis, in accordance with Dutch law the local medical ethical committee was informed about this research project and a waiver was granted (number METc2014.395).

WBC scintigraphy, acquisition and analysis

To properly analyze the results, all WBC scans were reassessed by an experienced nuclear medicine physician who was blinded for all clinical and microbiological results. All scans in the studied timeframe were performed using the same protocol. Radiolabeling of the autologous WBC (mixed leukocytes) with ^{99m}Tc-HMPAO was performed according to current EANM guidelines [11]. In accordance with recent insights on the correct acquisition and analysis of WBC scintigraphy dual time-point acquisition was used, with static images acquired 4 h and 20-24 h after intravenous injection of 370-555 MBq ^{99m}Tc-HMPAO-labelled WBC [10, 22]. The images were acquired with decay-corrected acquisition times and displayed and analyzed using a total counts intensity scale with the same intensity threshold, thus avoiding observer bias. A SPECT (40 sec per frame, 360° rotation) with low-dose CT scan was performed for precise location in all patients in whom uptake was seen on the 4 h images. All scans were performed on a SPECT/CT gamma camera system (Symbia™ T, Siemens Medical Systems, Knoxville, TN, USA). Scans were visually classified as negative for infection when 1) there was no uptake at all on both images, 2) when the uptake decreased in time, or 3) when the uptake remained the same in time. Scans were visually classified as positive for infection when the uptake showed an increase in size and/or intensity in time. In equivocal cases, semi-quantitative analysis was performed by drawing a region of interest (ROI) over the suspected infectious focus and an automatically mirrored ROI over contralateral reference tissue. The mean counts per pixel in these ROIs were recorded and a lesion-to-reference tissue (L/R) ratio was calculated on both images. When the L/R ratios decreased or remained stable in time, the scan was considered negative for infection. When the L/R ratios increased in time, the scan was considered positive for infection. Hence only the planar

images were used for declaring a scan positive or negative for infection, first visually and in equivocal cases semi-quantitatively. When a scan was considered positive for infection, SPECT/CT was used to determine if the infection was located in the bone (FRI) or outside the bone (soft-tissue infection). A clinical example of a WBC-scintigraphy + SPECT/CT for diagnosing a FRI is presented in Figure 1.

Statistical analysis

Either descriptive statistics (mean and standard deviation (SD) or median and range, frequency and percentages) were used to describe the characteristics of the study population. The discriminative ability of the imaging modalities was quantified by several measures of diagnostic accuracy: sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (PLR and NLR), and the diagnostic odds ratio (DOR).

To assess whether a variable was predictive of a false WBC scintigraphy test result (false-positive or false-negative versus true-positive or true-negative), a multivariable logistic regression analysis was performed. The following variables were entered into the model: duration of the interval between last surgery before WBC scintigraphy and the actual WBC scintigraphy itself, diabetes, obesity, smoking, drug use, and NSAID treatment or antibiotic treatment at time of WBC scintigraphy. The interval between last surgery and WBC scintigraphy was categorized into 0-3 months, 3-6 months and >6 months. A backward stepwise selection procedure was used, with Akaike's information criterion ($P < 0.157$) as selection criterion. Odds ratios (OR) with 95% confidence intervals were calculated. An OR >1 indicated that patients with a specific variable (for instance, obesity) had higher odds of having a false WBC scintigraphy test result than patients without the presence of that variable. Additionally, two similar models were constructed to assess whether the aforementioned variables were predictive of a specific false test result (false-positive versus true-positive, and false-negative versus true-negative). Statistical analyses were performed using IBM Statistics for Windows software (SPSS version 23.0, Armonk, NY: IBM Corp.).

Source of funding

No external funds were received in support of this study.

RESULTS

During the inclusion period a total of 192 WBC scintigraphies were performed in 162 patients who met the inclusion criteria. All WBC scintigraphies were requested for actual or suspected peripheral FRI. The patient characteristics are summarized in Table 1. The fracture specifics are presented in Table 2, the type of index operation in Table 3.

Table 1. Patient characteristics (N=162).

Sex[a]	
Male	104 (64)
Female	58 (36)
Age (years) [b]	50.4 (12.2 -87.3)
BMI*[b] (N=141)	28.5 (18.2- 46.3)
ASA classification[b] (N=146)	1.8 (1-3)
I [a]	55 (38)
II [a]	68 (47)
III [a]	23 (16)
Comorbidities and other risk factors[a]	
Diabetes	23 (14)
Psychiatric disorder	14 (9)
Obesity	25 (15)
(Pre-)malignant disorder	4 (3)
Anemia	1 (1)
Hypothyroidism	6 (4)
Smoking (N=143)	52 (36)
NSAIDs***	21 (13)
Corticosteroids	7 (5)
Recreational drugs	7 (4)
Cannabis	3 (2)
Heroin/methadone	4 (3)
Antibiotic treatment	17 (10)

[a] Data presented as N (%). [b] Data presented as mean (range).
* BMI: Body Mass Index, **ASA: American Society of Anaesthesiologists, ***NSAIDs: Non-Steroidal Anti- Inflammatory Drugs.

Table 2. Fracture characteristics

Fracture type according to Müller AO classification (N=192) [19]	
1: Humerus	**18**
11: Proximal	4
12: Diaphyseal	4
13: Distal	10
2: Radius/ulna	**9**
21: Proximal	3
22: Diaphyseal	6
23: Distal	0
3: Femur	**50**
31: Proximal	10
32: Diaphyseal	27
33: Distal	12
34: Patella	1
4: Tibia/fibula	**100**
41: Proximal	11
42: Diaphyseal	40
43: Distal	20
44: Malleolar	29
8: Foot	**15**
Soft tissue injury (N=192)	
Closed Fracture	102
Open fracture	90
Gustilo-Anderson Classification (N=90)[21]	
Type 1	17
Type 2	24
Type 3A	27
Type 3B	4
Type 3C	5
Unknown	13

N = total number of WBC scintigraphies included for analysis.

Table 3. Index operation (N=192)

Intramedullary nail		45 (23)
Plate		69 (36)
Screw(s)		7 (4)
Dynamic hip screw		7 (4)
External fixator		44 (23)
Followed by		
Intramedullary nail	10	
Plate	19	
Screw(s)	2	
Remaining cast	4	
Remaining external fixator	3	
Other	6	
Closed reduction		10 (5)
Managed conservatively		2 (1)
Other		3 (2)
Unknown		5 (3)

Data presented as N (%)

In 51% of cases (77 patients with 97 WBC scintigraphies) an adequate microbiological result was available. This cohort had a mean clinical follow-up of 18.3 months (SD 15.3), and 44 patients had an MMB-confirmed infection. Staphylococci were the most commonly identified organism **(Table 4),** with MRSA in two patients. Two patients had negative cultures but they both had a clear clinical FRI with a chronic and with the implant communicating fistula. Both patients were operated on by an experienced orthopedic surgeon specialized in PJI and FRI who diagnosed the FRI based on intraoperative assessment. The WBC scintigraphies for both these patients were positive and therefore regarded as true-positive. The patients without adequate MMB results (85 patients with 95 WBC scintigraphies) had a mean clinical follow-up of 21.9 months (SD 17.1). In this group 8 additional patients had a clinically detected infection during follow-up.

Table 4. Microbiological results of intraoperative collected tissue samples

Etiology of infection	Results of 44 culture-positive patients	Results of 9 culture-positive patients with false-negative WBC
Staphylococcus spp	28	6
Corynebacterium spp	4	2
Enterococcus spp	5	1
Proprioni spp	5	2
Streptococcus spp	3	1
E Coli	3	1
Pseudomonas spp	2	
Actinomyces spp	1	
Peptoniphilius spp	4	
Klebsiella spp	2	
Finegoldia spp	1	
Proteus spp	1	
Dermabacter spp	1	
Bacteroides spp	1	
Serratia spp	1	1
Polymicrobial	14 (32)	5 (56)

Data presented as N (%)

Overall there were 52 cases with an FRI; the WBC scintigraphy detected this correctly 41 times (TP). There was no FRI 140 times, as confirmed by WBC scintigraphy in 136 cases (TN). Four scans were false positive (FP) and 11 scans were false negative (FN). This resulted in 0.79 sensitivity, 0.97 specificity, 0.91 PPV, 0.93 NPV, 0.92 diagnostic accuracy, 126.7 DOR, 26.3 PLR and 0.22 NLR for the WBC scintigraphy to detect an FRI in the peripheral skeleton. Multivariable logistic regression analyses showed that only obesity was predictive of a false WBC scintigraphy test result (P=0.008, OR=5.42). The median BMI of the 4 patients with FP results was 36.0 kg/m^2 (range 29.7 – 37.4), of the 11 patients with FN results 29.4 kg/m^2 (range 24.8 – 35.6) and of the cohort patients with true results (TP + TN) 27.7 kg/m^2 (range 18.2 – 46.3) respectively. The duration of the interval between the operation and the WBC scintigraphy did not influence its diagnostic accuracy. In the group of WBC-scintigraphies performed within 0-3 months after the last surgery there were 15 correct (TP + TN) and zero incorrect (FP + FN) scan results. In the group of WBC-scintigraphies performed within 3-6 months this number

was 37 and five, respectively, and in the group of WBC-scintigraphies performed six months of more after the last surgery this number was 124 and 10, respectively. Median interval between last surgery and WBC-scintigraphy was 10.7 months (range 0.2 – 490.2). There were 9 patients with an interval of more than 10 years (median 180.0 months, range 126.1 – 490.2), the accuracy results of these patients were 1 TP, 6 TN and 3 FN respectively. All other variables were discarded from the logistic regression models. The additional logistic regression analyses showed that obesity was mainly predictive of a false-positive WBC scintigraphy test result (p=0.035, OR=11.67). The results of the multivariate logistic regression analyses are presented in Table 5.

Table 5. Results of multivariable logistic regression analyses

Model*	Variable	Regression coefficient	P-value	OR (95% CI)
1. False test result vs true test result	Obesity	1.69	0.008	5.42 (1.55–19.00)
2. FP vs TP	Obesity	2.46	0.035	11.67 (1.19–114.90)
3. FN vs TN	Obesity	1.39	0.08	4.03 (0.87–18.75)

* True WBC scintigraphy test result was used as reference group. No other variables are reported, as they do not constitute significant predictors of a false test result.
Abbreviations: FP, false-positive; TP, true-positive; FN, false-negative; TN, true-negative; OR, odds ratio; CI, confidence interval.

False-Positive/False-Negative patient characteristics

There were 11 patients with a false-negative (FN) WBC scintigraphy. Nine patients had positive MMB results, the other two were detected during clinical follow-up. Nine of the 11 FN patients had low-grade infected nonunions of bones in different body regions. The radius and ulna were involved in one case, the femur in two cases, the tibia in five cases, and the fibula in one case. One 45-year-old man with an FN result presented with an erysipelas which eventually turned out to be a chronic FRI of the tibia 24 years after a fracture (initial treatment involved an external fixator). The last patient with an FN result presented with a non-healing wound at the lower leg due to a long-standing FRI of the fibula 70 years after a grenade injury. His interval between last surgery and WBC scintigraphy was over 37 years. Overall all the majority of patients with a false negative WBC scintigraphy (N=9, 82%) in these series had low-grade infected non-unions.

Four patients had a false-positive WBC scintigraphy. One of them was a 45-year-old female who was treated for a nonunion at one year after a grade-1 complicated femoral fracture that was initially treated with an intramedullary nail. At revision surgery the

cultures turned out to be negative despite a positive WBC scintigraphy. The second patient with an FP result was a 40-year-old female with a grade 3A complicated femoral fracture for which she was operated several times. She had a 12-cm bone defect and cancellous bone graft in situ, and was treated for a nonunion two years after the initial injury. The third patient with an FP result was a 35-year-old man who was treated for a nonunion of the tibia three years after a complicated lower extremity fracture. The last patient with an FP result was a 73-year-old woman who had a nonunion of the distal femur one year after a complicated femoral fracture that was initially treated with a plate osteosynthesis.

DISCUSSION

Based on the results of a large patient cohort, we report a high diagnostic accuracy (0.92) of WBC scintigraphy for FRI in the peripheral skeleton with a sensitivity of 0.79, a specificity of 0.97, a PPV of 0.91, and an NPV of 0.93. We found no difference in accuracy of previously performed WBC scintigraphies (between 0-3 months after surgery) compared to subsequent scans.

Nuclear imaging is evolving rapidly; therefore only recent studies can be compared with current practice. Since the turn of the millennium five comparable studies have been published on the accuracy of WBC scintigraphy for FRI. Only two of these studies utilize SPECT/CT as an adjunct to WBC scintigraphy in case of a positive result. This has shown to improve diagnostic accuracy, as it distinguishes whether the infection is situated in the bone or in soft tissue [10, 12, 23]. The specificity in our study is in line with the aforementioned studies investigating WBC scintigraphy in combination with SPECT/, yet the sensitivity in our study is lower. The reason is unclear even though the inclusion criteria in the two other studies are similar to ours. However, the strength of the current study is that it consists of a much larger consecutive series (our study consists of 192 WBC scintigraphies vs 49 [10] and 29 [23] WBC scintigraphies respectively).

Whether an imaging result can be trusted shortly after an operation or fracture, is important for a correct interpretation of the results. Especially regenerating bone and soft-tissue disruption is expected to have some impact on the results of a WBC scintigraphy, probably similarly to a bone scan, magnetic resonance imaging (MRI) and fluorodeoxyglucose positron emission tomography (FDG-PET) scan [5, 12]. In our study the only variable highly predictive for a false result was obesity. The reason for it is unknown and this phenomenon has not been reported earlier in the literature. We should however keep in mind that this result is based on a limited number of FN

(N = 11) and FP (N = 4) patients with the highest BMI in the group with FP results. Further prospective research is warranted to explain these findings. None of the other variables influenced the results of the WBC scintigraphy (Table 5). This means that a WBC scintigraphy is not only accurate shortly after an operation, but also with the concomitant use of recreational drugs, antibiotic treatment, smoking or comorbidities such as diabetes mellitus.

The limitations of this study, apart from its retrospective design, include the potential for selection bias as usually only patients with clinically difficult-to-detect FRIs undergo advanced nuclear imaging. It was not feasible to identify all patients with a low-grade FRI, but when we analyzed the FP and FN WBC scintigraphies we detected that nine out of the 11 patients with an FN scan result had the clinical appearance of a low-grade infected nonunion. One could argue that WBC scintigraphy might be less accurate in low-grade FRI due to lower leucocyte availability (hence the lower sensitivity). Unfortunately, this may be the patient population for whom a WBC scintigraphy is usually requested, therefore further prospective studies that include other imaging modalities such as MRI and FDG-PET are warranted.

CONCLUSION

In conclusion, this study indicates:

1) That WBC scintigraphy has a high diagnostic accuracy (0.92) for detecting fracture-related infections in the peripheral skeleton based – for the first time – on a large patient cohort.
2) That the time interval between WBC scintigraphy and previous surgery does not interfere with the results. This means that a WBC scintigraphy is accurate shortly after an operation.

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CHAPTER 6

The diagnostic accuracy of ^{18}F-FDG-PET/CT in diagnosing fracture-related infections

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ABSTRACT

Introduction. ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET/CT) is frequently used to diagnose fracture-related infections (FRI), but its diagnostic performance in this field is still unknown. This study aimed to (1) assess the diagnostic performance of qualitative ^{18}F-FDG-PET/CT assessment in diagnosing FRI, (2) establish optimal diagnostic performance of Standardized Uptake Values (SUVs) in ^{18}F-FDG-PET/CT and report associated cut-off values, and (3) identify variables that predict a false-positive (FP) or false-negative (FN) ^{18}F-FDG-PET/CT result.

Methods. This retrospective cohort study included all patients with suspected FRI undergoing ^{18}F-FDG-PET/CT between 2011 and 2017 in two level-I trauma centers. Two nuclear medicine physicians independently re-assessed all ^{18}F-FDG-PET/CTs. The reference standard consisted of at least 2 representative microbiological culture results or the presence/absence of clinical confirmatory FRI signs (AO/EBJIS consensus definition) during at least 6 month follow-up. Diagnostic performance in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was calculated. Additionally, SUVs were measured on ^{18}F-FDG-PET/CTs. Volumes-of-interest were drawn around the suspected- and corresponding contralateral area to obtain absolute values and ratios between suspected- and contralateral areas. A multivariable logistic regression was performed to identify the most important predictor(s) for FP or FN ^{18}F-FDG-PET/CT results.

Results. During the study period, 158 ^{18}F-FDG-PET/CTs were performed. Qualitative assessment of ^{18}F-FDG-PET/CT showed a sensitivity of 0.89, a specificity of 0.79, PPV of 0.73, NPV of 0.92 and diagnostic accuracy of 0.83. SUVs on their own resulted in lower diagnostic performance, but combining them with qualitative assessments yielded an AUC of 0.89, compared to an AUC of 0.84 when regarding only qualitative assessment results. ^{18}F-FDG-PET/CT performed < 1 month after surgery was found to be the most predictive independent variable for false test results, with an absolute risk of 46% (95% CI 29-65) compared to 9% (95% CI 5-14) in patients with an interval of 1-6 months.

Conclusion. Qualitative ^{18}F-FDG-PET/CT assessment has a diagnostic accuracy of 0.83 when diagnosing FRI. Adding SUV measurements to qualitative assessment provides additional accuracy in comparison to qualitative assessment alone. An interval between surgery and ^{18}F-FDG-PET/CT < 1 month was associated with a sharp increase in false test results.

INTRODUCTION

Fracture-related infection (FRI) is a serious complication following trauma surgery and can lead to increased morbidity and high medical costs [1, 2]. Due to the fact that clinical symptoms are not always evident, diagnosing FRI can be challenging. This problem was worsened by the fact that, until recently, no uniform definition of FRI existed [3]. Recently, the AO foundation and European Bone and Joint Infection Society (EBJIS) published a consensus definition comprising confirmatory- and suggestive criteria for diagnosing FRI [4]. Medical imaging is herein considered only to be an adjunct to the diagnosis of FRI (i.e. a suggestive criterion). The reason for this is that its evidence on the diagnostic accuracy for FRI is limited. Moreover, the scarcely available evidence is mainly obtained for studies dealing with other causes of bone infection such as diabetic feet, periprosthetic joint infection (PJI) and hematogenous osteomyelitis [5]. Most of the previous studies on diagnostic imaging for FRI were hampered by small patient cohorts, unclear reference standards and heterogeneous orthopedic patient populations [5, 6]. Recently, our group reported a high diagnostic accuracy of white blood cell scintigraphy (WBC scintigraphy) for diagnosing FRI of 0.92 [7]. To compare imaging modalities, we used the same study design to evaluate the diagnostic performance of ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT).

The three aims of the current study were:

1) to establish the diagnostic performance of qualitative ^{18}F-FDG-PET/CT assessment for diagnosing FRI.
2) to establish the diagnostic performance of Standardized Uptake Values (SUVs) in ^{18}F-FDG-PET/CT for diagnosing FRI and to report their optimal associated cut-off values.
3) to determine which variables are independent predictors of a false positive or false negative ^{18}F-FDG-PET/CT test result in patients suspected of FRI.

METHODS

Ethical approval

Due to the observational nature of this study a waiver was granted by the medical ethical review committee (METC) of the University Medical Center Utrecht [METC 17-475].

Study design and eligibility criteria

A retrospective, dual center cohort study was performed that included patients in two large level-1 trauma centers in the Netherlands: the University Medical Center Utrecht (UMCU) and University Medical Center Groningen (UMCG). All consecutive patients undergoing ^{18}F-FDG-PET/CT for diagnosing (or excluding) FRI between January 2011 and November 2017 were eligible for inclusion. FRI was considered as either an infection following an open fracture (irrespective of type of treatment), or an infection following fracture surgery, or an infection following instrumented fusion for spinal fractures. We excluded patients undergoing ^{18}F-FDG-PET/CT for other reasons than diagnosing FRI, like PJI, non-traumatic osteosyntheses or hematogenous osteomyelitis. Also, patients who did not comply with the reference standard were excluded.

Index test

The index test consisted of the ^{18}F-FDG-PET/CT. Scanning protocols were similar in both centers. Scans were acquired approximately 60 minutes after intravenous administration of 2-3 MBq/kg ^{18}F-FDG according to existing European Association of Nuclear Medicine (EANM) guidelines for ^{18}F imaging [8]. Scans were acquired on either a Biograph mCT64 slice- or a Biograph mCT40 slice PET-CT system (Siemens, Knoxville, TN, USA).

After anonymization, the scans were independently re-assessed by two experienced nuclear medicine physicians (MGGH and AWJM). Both nuclear medicine physicians were blinded to the reference test result. Nuclear imaging signs such as uptake location, uptake pattern (multifocal, heterogeneous, diffuse homogenous), uptake grade (0: no uptake, 1: higher uptake in the alleged infected side compared to contralateral side, 2: much higher uptake in the suspected infected side compared to contralateral side), involvement of osteosynthesis material and soft tissue- and bone involvement, were documented for each of the scans in a case record form (CRF). Disagreements were resolved through discussion until consensus was reached. A clinical case example of an ^{18}F-FDG-PET/CT for FRI is provided in Figure 1.

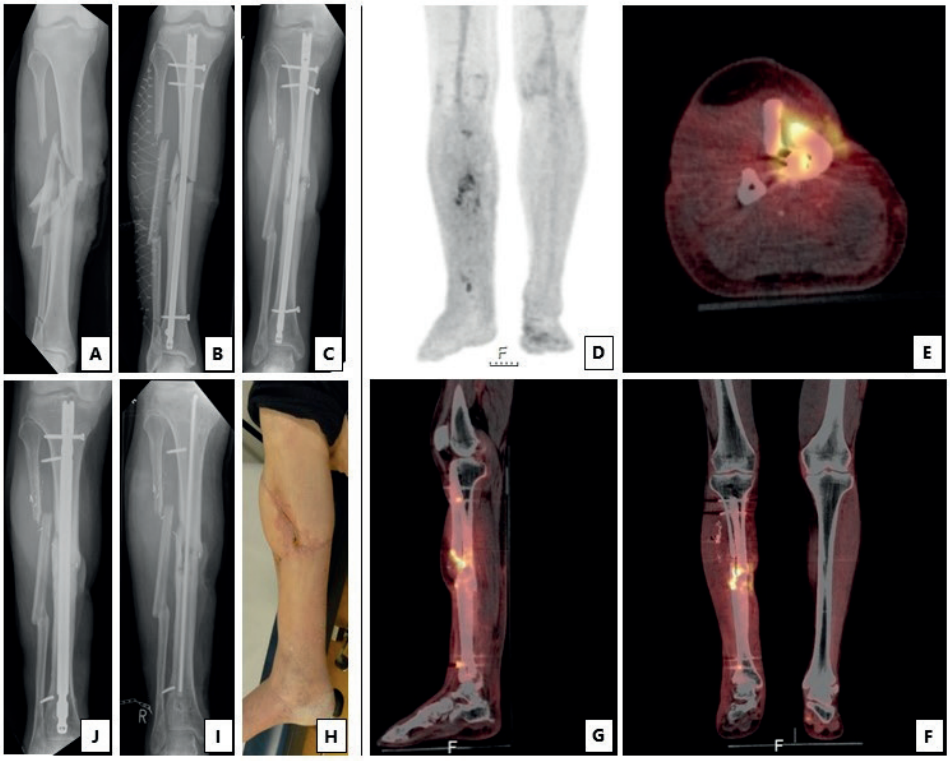

Figure 1. Clinical case. A 59-year-old man sustained a right sided Gustillo grade III-B open crural fracture **(A)** which was treated with intramedullary nailing **(B)** and a fasciotomy. After several soft tissue debridements, the remaining soft tissue defect was eventually closed with a free musculocutaneous flap. Twenty months later, there was a non-union with "auto-dynamisation" of the intramedullary nail, demonstrated by the broken interlocking screws **(C)**. ^{18}F-FDG-PET scan showed increased uptake around the fracture site in the tibial shaft and around the proximal and distal screws **(D)**. Fusion ^{18}F-FDG-PET/CT images localized the suspected fracture-related infection (FRI) to be not only at the fracture side but also in the surrounding bone of the tibia around the fracture site **(E: Axial, F: Coronal, G: Sagittal)** which corresponded with the unstable scar overlapping the area of the non-union **(H)**. The intramedullary nail was removed, the tibia was reamed, the fracture site was debrided and an in-house, custom-made antibiotic nail was inserted. FRI was confirmed by microbiological cultures and the patient was subsequently treated with antibiotics. One year after exchange nailing, fracture healing was successful **(J).**

Semi-quantitative measurements in the form of SUVs were also measured on all EANM Research Ltd (EARL) reconstructed ^{18}F-FDG-PET/CT scans. SUVs correspond to the extent of ^{18}F-FDG uptake and consequently, to cellular glucose metabolism. Because glucose metabolism is increased in infected cells, higher measured SUVs correspond to a greater risk of FRI compared to lower SUVs.[9] SUVs were gathered by drawing a spherical volume of interest (VOI) in both the suspected infected target area and an anatomical corresponding area on the contralateral side as reference. Additionally, a VOI was drawn in nearby muscle for background comparison. For all VOIs, both SUV_{max} (single-pixel value) and SUV_{peak} (average value in a high-uptake part of the VOI) were calculated. For SUV_{max} and SUV_{peak}, the ratios between the suspected infected side and contralateral side were also calculated. To correct for background ^{18}F-FDG uptake, we calculated ratios between SUVs on the suspected infected site and SUVs in nearby muscles ($SUV_{maxmuscleratio}$ and $SUV_{peakmuscleratio}$). These data were reported in a separate case record form as continuous measurements. All SUV measurements were corrected for body weight and blood glucose level and were performed with Syngo.via software (Siemens Healthineers, Forchheim, Germany).

Reference test

The final diagnosis of FRI (reference test) was based on the outcome of medical microbiological (MMB) culture results in case of surgical intervention, or – if unavailable – on clinical follow-up of at least six months. Because this study retrospectively analyses culture results in an era when no uniform culturing protocol existed, we applied strict criteria when judging the validity of the reference test. All MMB results were judged by an experienced trauma surgeon on their validity to detect FRI. The microbiological results of swabs and cultures of fistulas were disregarded due to relatively low accuracy [10-12]. The MMB results were only considered representative if at least two surgically obtained deep-tissue cultures were available from the suspected infectious site. A positive FRI result was defined as at least two positive representative MMB cultures with the same micro-organism according to the microbiological criteria of the AO/EBJIS consensus definition [4]. FRI during clinical follow-up was defined according to the clinical confirmatory criteria set forth in the AO/EBJIS consensus definition as any wound breakdown, purulent drainage or the presence or development of a sinus tract (communicating with the implant material) [4]. If culture results were negative but there were confirmatory FRI criteria (e.g. pus, fistula) per-operatively when cultures were taken, FRI was deemed to be present (and the culture result was considered to be erroneous). These culture-negative FRIs are known to be caused by low virulent bacteria like coagulase negative staphylococcus species [13].

Statistical analyses

To assess the diagnostic performance of the ^{18}F-FDG-PET/CT scan, the number of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) test results were obtained. From this, sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV), positive- and negative likelihood ratios and diagnostic odds ratios with 95% confidence intervals (CIs) were calculated.

All standardized uptake values were compared between groups with student t-tests (if normally distributed) or Mann-Whitney U tests (if not normally distributed). Normality of the data was tested through visual inspection of the normality plots. The sensitivity and specificity of the separate SUV measurements were plotted in receiver operating characteristic (ROC) curves and for each curve, area under the curve (AUC) was calculated. The Q-point on each curve (i.e. the point which maximizes both sensitivity and specificity) was determined and the associated cut-off value was extracted. In addition, an ROC curve was plotted combining the diagnostic performance of both SUV measurements as well as qualitative assessment and the associated AUC was calculated.

Consequently, a backward stepwise multivariable logistic regression analysis was performed to determine which variables were independent predictor(s) of a false (i.e. false positive or false negative) test result. Removal testing was performed with the probabilities of the likelihood-ratio statistic based on the maximum partial likelihood estimates. Multiple variables that are suggested to influence ^{18}F-FDG-PET/CT accuracy in the literature were included into the model [14]. The variables entered were: interval between last operative procedure (or trauma date if no operation was performed) and ^{18}F-FDG-PET/CT (ordinal: < 1 month, between 1-6 months and > 6 months), BMI (continuous), diabetes mellitus (dichotomous), smoking history (dichotomous), non-steroidal anti-inflammatory drug (NSAID) use at time of ^{18}F-FDG-PET/CT (dichotomous) and antibiotic use at time of ^{18}F-FDG-PET/CT (dichotomous). Using the final model, probabilities of false test results were obtained (with 95% CIs) for the different variables. Additionally, diagnostic performance was calculated when excluding cases with a high risk of a false test result. All statistical analyses were performed with SPSS statistics v 22.0 (IBM, Chicago, Illinois, USA).

RESULTS

In the study period, a total of 136 patients undergoing 158 ^{18}F-FDG-PET/CTs were ultimately included. The patient characteristics are summarized in Table 1. The fracture specifics are presented in Table 2, the type of index operation in Table 3.

Table 1. Baseline characteristics.

Age (years)[a]	46.2 (4-76)
Sex (male)[b]	113 (71.5)
BMI (kg/m^2)[a]	27.0 (15.3-48.1)
ASA score (%)[b]	
1	59 (37.3)
2	73 (46.2)
3	10 (6.3)
4	1 (0.6)
Unknown	15 (9.5)
ISS[b]	
< 16	93 (58.9)
≥ 16	58 (36.7)
Unknown	7 (4.4)
Comorbidities/Risk factors at time of ^{18}F-FDG-PET/CT[b]	
Diabetes mellitus	16 (10.1)
Psychiatric disease	15 (9.5)
Obesity	31 (19.6)
Hypothyroidism	4 (2.5)
Hypertension	19 (12.0)
Tobacco abuse	63 (39.9)
Alcohol abuse	11 (7.0)
Drug abuse	9 (5.7)
NSAID use	34 (21.5)
Corticosteroid use	3 (1.9)
Antibiotic use	35 (22.2)

[a] Data presented as mean (range), [b] Data presented as N (%), BMI: Body mass index, ISS: Injury severity score, ASA: American society of anaesthesiologists, NSAID: Nonsteroidal anti-inflammatory drug.

Table 2. Fracture Characteristics.

AO fracture classification	
1: Humerus	5 (3.2)
13: Distal	1 (0.6)
15: Clavicle	4 (2.5)
2: Radius/Ulna	8 (5.1)
21: Proximal	3 (1.9)
22: Diaphyseal	3 (1.9)
23: Distal	2 (1.3)
3: Femur	26 (16.5)
31: Proximal	1 (0.6)
32: Diaphyseal	19 (12.0)
33: Distal	6 (3.8)
4: Tibia/Fibula	89 (56.3)
41: Proximal	12 (7.6)
42: Diaphyseal	49 (31.0)
43: Distal	16 (10.1)
44: Malleolar	12 (7.6)
5: Spine[a]	14 (8.9)
A: Compression injury	9 (5.7)
B: Distraction injury	1 (0.6)
C: Dislocation injury	3 (1.9)
Unknown	1 (0.6)
6: Pelvis/Sacrum	5 (3.2)
8: Foot	11 (7.0)
81: Talus	3 (1.9)
82: Calcaneus	6 (3.8)
83: Navicular	1 (0.6)
Unknown	1 (0.6)
Closed/Open fractures[b]	
Closed fractures	69 (43.7)
Open fractures	77 (48.7)
Type I	13 (8.2)
Type II	11 (7.0)
Type IIIA	20 (12.7)
Type IIIB	6 (3.8)
Type IIIC	3 (1.9)
Unknown	24 (15.2)
Unknown	12 (7.6)

Data presented as N (%), AO: Arbeitsgemeinschaft für Osteosynthesefragen, [a] AO Spine Injury Classification, [b] Gustillo-Anderson classification.

Table 3. Index procedure data.

Operative	151 (95.6)
Plate	54 (34.2)
Screw(s)	16 (10.1)
Intramedullary nail	35 (22.2)
Arthrodesis (including spinal fusion)	14 (8.9)
Amputation	1 (0.6)
External fixator followed by:	31 (19.6)
Plate	17 (10.8)
Screw	1 (0.6)
Intramedullary nail	5 (3.2)
Conservative	2 (1.3)
Unknown	6 (3.8)
Closed reduction/conservative	5 (3.2)
Unknown	2 (1.3)

Data presented as N (%).

In 43% of cases (68 ^{18}F-FDG-PET/CTs), a representative microbiological result was available. This cohort had a median clinical follow-up of 13.8 months (IQR 20.6), 33 of these patients (49%) had an MMB-confirmed FRI. Staphylococcus species were most commonly cultured (Table 4). There were 11 cases in which culture results were negative but there were per-operative confirmatory signs of FRI, like purulent drainage, wound breakdown or a fistula communicating with implant material. These cases were scored as positive for FRI.

The 57% of cases without MMB results (90 ^{18}F-FDG-PET/CTs) had a median clinical follow-up of 16.3 months (IQR 23.9). In this group, 18 cases showed clinical confirmatory signs of FRI, the other 72 cases had an uneventful clinical follow-up.

In total, 62 cases were diagnosed with FRI. In 55 out of 62 cases, the ^{18}F-FDG-PET/CT was positive for FRI (TP). Ninety-six cases were regarded to be negative for FRI. In 76 out of 96 cases, the ^{18}F-FDG-PET/CT correctly ruled out an FRI (TN). There were 20 FP and 7 FN cases. This resulted in a sensitivity of 0.89 (95% CI 0.78-0.95), specificity of 0.79 (95% CI 0.70-0.87), positive predictive value of 0.73 (95% CI 0.65-0.80), negative predictive value of 0.92 (95% CI 0.84-0.96), positive likelihood ratio of 4.26 (95% CI 2.85-6.35), negative likelihood ratio of 0.14 (95% CI 0.07-0.29), and a diagnostic odds ratio of 29.9 (95% CI 11.8-75.5). The diagnostic accuracy of ^{18}F-FDG-PET/CT for diagnosing FRI was 0.83 (95% CI 0.76-0.88).

Table 4. Microbiological data.

Positive culture results (N=33)	True positive (N=31)	False negative (N=2)
Staphylococcus aureus	12	1
Coagulase negative staphylococcus spp.	10	
Streptococcus spp.	4	
Corynebacterium spp.	2	
Enterococcus spp.	4	
Finegoldia magna		1
Actinomyces neuii	1	
Propionibacterium acnes	1	
Pseudomonas aeruginosa	4	
Escherichia coli	2	1
Enterobacter cloacae	2	
Serratia marcescens	1	
Fusobacterium gonidiaformans	1	
Bacteroides thetaiotaomicron	1	
Proteus vulgaris	1	
Klebsiella oxytoca	1	
Morganella morganii	1	
Bacteroides fragilis	1	
Polymicrobial	11	1

Data presented as N (%).

Semi-quantitative measurements

Semi-quantitative measurements are presented in Table 5. Patients with FRI had a median SUV_{max} of 5.9 (IQR 3.6) and median SUV_{peak} of 4.7 (IQR 2.4) in the suspected infected area. Patients without FRI had a median SUV_{max} of 3.2 (IQR 2.5) and a median SUV_{peak} of 2.6 (IQR 1.9) in the area which initially was suspected for infection. These differences between both groups were significant, both for SUV_{max} ($p < 0.001$) as well as for SUV_{peak} ($p < 0.001$). The ratios found by dividing the SUVs in the suspected infected area by the SUVs in the contralateral area were 3.1 (IQR 2.2) for SUV_{max} and 2.9 (IQR 2.0) for SUV_{peak} in FRI patients. Patients without FRI had ratios of 1.9 (IQR 1.4) and 1.8 (IQR 1.4) for SUV_{max} and SUV_{peak} respectively. Both ratios were significantly different between FRI and non-FRI patients ($p<0.001$). Ratios between SUVs in the suspected infected area and SUVs in nearby muscle were 6.4 (IQR 4.9) for SUV_{max} and 5.5 (IQR 3.6) for SUV_{peak} in

FRI patients. For FRI negative patients, these ratios were 3.5 (IQR 2.8) and 3.2 (IQR 2.7) for SUV_{max} and SUV_{peak} respectively. These ratios were also significantly different between FRI and non-FRI patients (P<0.001).

Table 5. Semi-quantitative measurement data.

	All patients (N = 157)[a]	Patients with FRI (N = 60)	Patients without FRI (N =97)	p value
^{18}F-FDG dose (MBq)	193.0 (IQR 78.0)	199.5 (IQR 131.0)	190.0 (IQR 73.0)	0.020
Blood glucose (mmol/l)	5.6 (IQR 1.1)	5.7 (IQR 0.8)	5.5 (IQR 0.8)	0.696
SUV_{max} infection location	4.2 (IQR 3.4)	5.9 (IQR 3.6)	3.2 (IQR 2.5)	< 0.001
SUV_{max} contralateral location	1.7 (IQR 0.7)	1.8 (IQR 1.0)	1.7 (IQR 0.7)	0.037
$SUV_{maxratio}$[b]	2.1 (IQR 1.8)	3.1 (IQR 2.2)	1.9 (IQR 1.4)	< 0.001
$SUV_{maxmuscleratio}$[b]	4.6 (IQR 4.0)	6.4 (IQR 4.9)	3.5 (IQR 2.8)	< 0.001
SUV_{peak} infection location	3.5 (IQR 2.7)	4.7 (IQR 2.4)	2.6 (IQR 1.9)	< 0.001
SUV_{peak} contralateral location	1.4 (IQR 0.7)	1.5 (IQR 0.7)	1.4 (IQR 0.7)	0.060
$SUV_{peakratio}$[b]	2.1 (IQR 1.8)	2.9 (IQR 2.0)	1.8 (IQR 1.4)	< 0.001
$SUV_{peakmuscleratio}$[b]	4.0 (IQR 3.4)	5.5 (IQR 3.6)	3.2 (IQR 2.7)	< 0.001

Data presented as median (+ interquartile range; IQR), FRI: Fracture related infection

[a] SUV measurements could not be retrieved in 1 patient due to technical reasons

[b] Ratios were calculated by dividing value of suspected infected area by value of contralateral area/nearby muscle; a value > 1 signifies higher uptake in suspected infected area

The semi-quantitative data were plotted in ROC curves (Figure 2). Area's under the curve were 0.80 (95% CI 0.73-0.87) for SUV_{max}, 0.73 (95% CI 0.64-0.81) for $SUV_{maxratio}$ and 0.77 (95% CI 0.69-0.85) for $SUV_{maxmuscleratio}$. Optimal sensitivity and specificity for SUV_{max} were 0.80 and 0.71 at a cutoff of 4.2. For $SUV_{maxratio}$, sensitivity was 0.58 and specificity was 0.81 at a cutoff of 2.9 and for $SUV_{maxmuscleratio}$, sensitivity was 0.70 and specificity was 0.71 at a cutoff of 4.8. The diagnostic parameters and associated cut-off values for the SUV_{peak} measurements were similar to the SUV_{max} measurements and can be found in Figure 2.

Combining the SUV measurement data with the qualitative ^{18}F-FDG-PET/CT assessments in a separate ROC curve yielded an AUC of 0.89 (95% CI 0.84-0.95) and diagnostic accuracy of 0.87 (sensitivity 0.85, specificity 0.87), as opposed to the AUC of 0.84 (95% CI 0.77-0.91) and diagnostic accuracy of 0.83 when regarding only the qualitative assessment result.

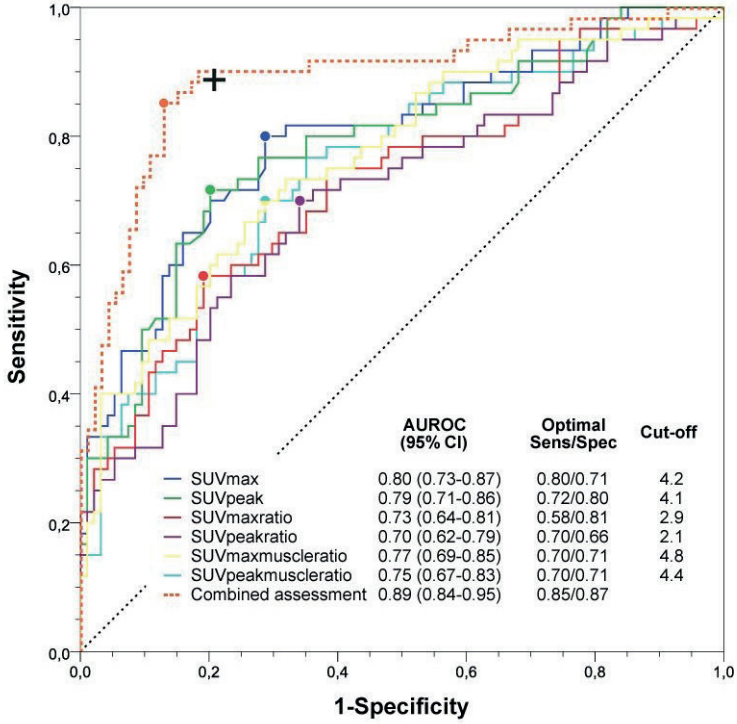

Figure 2. Receiver operating characteristics (ROC) curve. The diagnostic performance of the different semi-quantitative measurements can be seen and compared. The Q-points on respective curves represent the optimum between sensitivity and specificity at a specific cut-off value, these can also be found in the figure. It can be seen that the combined sensitivity and specificity of the qualitative nuclear medicine specialist assessment (represented by the black cross) is higher than the Q-point of any of the semi-quantitative measurements on their own. When combining qualitative and semi-quantitative assessment, AUROC increases to 0.89.
AUROC: Area under the receiver operator characteristics curve, Sens: Sensitivity, Spec: Specificity.

False Negative/False positive patient characteristics

Seven patients were included with a false negative test result. Two patients had positive intra-operative cultures, while five patients showed confirmatory signs per-operatively or during the 6 months of follow-up. Two patients had (low-grade) infected non-unions (both ankle fractures). Another patient (with two scans) showed per-operative signs of FRI in the tibia (infected tissue and pus), despite microbiological cultures remaining negative.

There were 20 patients with a false positive test result. These included 2 lower arm fractures, 3 femoral fractures, 2 tibial plateau fractures, 7 lower leg fractures, 2 ankle fractures, 2 talar fractures and 2 spinal fractures. Nine patients had negative intra-operative cultures, 11 had no cultures taken, but showed an uneventful 6 month follow-up without signs of FRI. Five cases (25%) with a false positive result were operated in the week before the ^{18}F-FDG-PET/CT (1 tibial fracture, 1 talar fracture, 1 ankle fracture, 2 tibial plateau fractures). These scans were performed in known FRI positive cases (at the time of the scan) to ascertain whether FRI had receded or was still advancing.

Predictors false test results

The most important predictor for a false test result was an interval between last operative procedure and ^{18}F-FDG-PET/CT of less than 1 month (B = 2.173; intercept = -2.327). The associated absolute predicted risk of a false result with this variable was 46% (95% CI 29-65) compared to an absolute predicted risk of the reference group (interval 1-6 months) of 9% (95% CI 5-14). For the patients with an interval > 6 months, the absolute risk was 17% (95% CI 10-27). Six out of 14 patients (42.9%) undergoing ^{18}F-FDG-PET/CT within 1 month received an erroneous test result, this was a false positive result in all six cases. In the period between 1-6 months, this rate declined to 5 out of 47 results (10.6%), and in the period after 6 months the rate slightly increased to 16 out of 95 results (16.8%). If the results from the early ^{18}F-FDG-PET/CTs (performed within 1 month after surgery) are omitted, diagnostic accuracy of qualitative assessment rises to 0.85 (95% CI 0.79-0.91) and sensitivity and specificity become 0.88 (95% CI 0.76-0.95) and 0.84 (95% CI 0.75-0.91) respectively.

DISCUSSION

The current study shows that qualitative assessment of ^{18}FDG-PET/CT for diagnosing FRI has good diagnostic performance with a diagnostic accuracy of 0.83 and an AUC of 0.84 (95% CI 0.77-0.91). Combining results of both qualitative assessment and SUV measurements resulted in an even higher diagnostic accuracy (0.87) and AUC (0.89 (95% CI 0.84-0.95)), which shows that including SUV measurements provides additional diagnostic accuracy, although the increase in accuracy is relatively small.

The sensitivity and specificity rates

in our results are in line with other studies reporting on the accuracy of ^{18}F-FDG-PET/CT in diagnosing FRI [5, 9]. However, the current study also included semi-quantitative measurements and used strict ^{18}F-FDG-PET/CT assessment- and reference test criteria (based on the recently released AO/EBJIS consensus definition of FRI) [4]. It is also

the largest series of FRI suspected patients to date undergoing hybrid ^{18}F-FDG-PET/CT imaging. One systematic review and meta-analysis on the diagnostic accuracy of different imaging modalities for chronic osteomyelitis showed higher diagnostic accuracy of ^{18}F-FDG-PET with a pooled sensitivity of 0.96 and specificity of 0.91 [6]. This study, however, only included studies published before 2003 and investigated only ^{18}F-FDG-PET without fusion CT images, which is rarely used anymore since the advent of ^{18}F-FDG-PET/CT scanners. In addition, reference test criteria were unclear in some of the included studies and the included studies included few patients and a relatively large number of spinal ^{18}F-FDG-PET/CTs. A more recent systematic review found sensitivities ranging between 0.86-0.94 and specificities of 0.76-1.00 of the ^{18}F-FDG-PET/CT for diagnosing FRI [5]. These results, as well as the used methodology (patient population and reference standard) are comparable to our study.

There is only limited research on the diagnostic accuracy of quantification in diagnosing FRI. A recent study on ^{18}F-FDG-PET/CT performing SUV measurements for diagnosing FRI found a sensitivity of 0.65 and specificity of 0.77 at a SUV_{max} of 4.0 [15]. These diagnostic parameters are lower than earlier published research on qualitative assessment of ^{18}F-FDG-PET/CT accuracy [5]. The reason for this could be that this SUV measurement study only used ^{18}F-FDG-PET/CT to differentiate between infected non-unions and aseptic non-unions. In both circumstances an increased bone metabolism will often be found and thus, differences between ^{18}F-FDG uptake will be limited. The cut-off of 4.0 that these authors reported is similar to the SUV_{max} cut-off found in our current study (4.2). Unfortunately, the validity of the results of their study is difficult to compare to our study, because it is unclear whether the authors used the standardized EARL scanning protocols [16]. Additionally, only semi-quantitative measurements, and no qualitative criteria (such as uptake pattern- and grade) for diagnosing FRI were used. SUV measurements do not take into account the activity pattern and uptake location and can be positive as a consequence of both bone healing and/or non-union. Therefore, using only semi-quantitative data might misclassify some patients. This is supported by our own results, in which the diagnostic accuracy of the qualitative assessment by the nuclear medicine physicians is higher than the accuracy of using SUVs alone. This phenomenon was also shown by a large study of FRI patients which demonstrated a diagnostic accuracy of 0.82 with qualitatively assessment of the ^{18}F-FDG-PET(/CT) and a lower accuracy when only regarding semi-quantitative measurements (SUV_{max}: sensitivity: 0.69, specificity 0.66 at cut-off 3.9) [9]. Another study investigating SUVs in histologically proven culture-positive and culture negative FRI patients showed that SUVs in both types of FRIs were similar (SUV_{max}: culture-pos 3.73, culture-neg 2.81) [17].

These studies, as well as the current study add to the mounting evidence that semi-quantitative measurements can be used as additional diagnostic tools in diagnosing FRI.

White blood cell scintigraphy is better investigated as a diagnostic imaging modality for diagnosing FRI. Our previously reported study on the diagnostic accuracy of WBC scintigraphy found a diagnostic accuracy of 0.92, which is higher than the diagnostic accuracy found in the current study for ^{18}F-FDG-PET/CT (0.83) [7]. However, ^{18}F-FDG-PET/CT does have several advantages over WBC scintigraphy. First, there is no need for manipulation of white blood cells, which is a laborious and expensive part of WBC scintigraphy [18]. Second, ^{18}F-FDG-PET/CT can be performed much quicker (one hour following radionuclide injection) and takes only one scanning session, as opposed to WBC scintigraphy, which takes at least two scans (4 hours and 20-24 hours after radionuclide injection) in two consecutive days [18]. Third, WBC scintigraphy has a reduced accuracy when used for diagnosing infections in the axial skeleton due to physiological uptake in the bone marrow, while ^{18}F-FDG-PET/CT does not have this limitation [14]. ^{18}F-FDG-PET/CT has the disadvantage that implants negatively impact diagnostic accuracy, although in some studies, this effect has not been shown [5, 9]. Ultimately, both imaging modalities have their specific advantages and limitations and although ^{18}F-FDG-PET/CT has lower accuracy than WBC scintigraphy, its advantages in logistics and patient comfort makes it a good alternative to WBC scintigraphy as the first nuclear imaging modality to perform when diagnosing FRI. As such, both modalities can be used to diagnose FRI depending on physician/hospital preference, financial considerations, and/or experience with either technique.

We found that an ^{18}F-FDG-PET/CT performed < 1 month following surgery was correlated to a false ^{18}F-FDG-PET/CT test result. It is known that operative procedures cause tissue damage and inflammation/regeneration, which shows increased uptake on ^{18}F-FDG-PET/CT, especially when the interval between the ^{18}F-FDG-PET/CT and surgery is short [14]. Five of the false positive ^{18}F-FDG-PET/CT scans were performed within a week after an operative procedure. Both nuclear medicine physicians re-assessing these scans for this study agreed that in some of these cases, inflammation due to surgery was indistinguishable from FRI. We may conclude that ^{18}F-FDG-PET/CT should therefore not be performed as a diagnostic tool within a month from surgery. If (per protocol) early (<1 month after surgery) ^{18}F-FDG-PET/CTs for FRI are no longer performed, diagnostic accuracy increased from 0.83 to 0.85.

Strengths of the current study are the large cohort size, and the fact that we performed a robust, standardized and repeatable scan assessment with 2 independent nuclear

medicine physicians (one from each hospital) who were blinded to the reference standard. We also used strict reference standard criteria to determine whether FRI was present or not, based on the recently published consensus definition. Finally, the addition of SUV measurements and SUV analysis provides additional insight on its merits, and shows how it performs compared to standard qualitative assessments.

Limitations of the current study include its retrospective design, with the associated risks of selection- and differential misclassification bias. Patients were recruited in two different teaching hospitals, which may have led to differences in either the diagnostic work-up or treatment of FRI as each hospital has their own standard of care. Also, in some patients, FRI had already been diagnosed and the ^{18}F-FDG-PET/CT scans were used for treatment follow-up. This mainly occurred in the beginning of the study period; since then, stricter protocols have been adopted, which aim to standardize both ^{18}F-FDG-PET/CT indications as well as microbiological culture acquisition and treatment regimes. Finally, it is important to remember that the combined assessment by two nuclear medicine specialists might lead to a higher diagnostic accuracy than can be obtained in the normal clinical situation, where only one nuclear medicine physician reviews a scan. Further prospective studies are warranted that will compare different imaging modalities for diagnosing FRI.

CONCLUSION

This study concludes that:

1) ^{18}F-FDG-PET/CT has a good diagnostic accuracy (0.83) for diagnosing FRI.
2) SUV measurements provide additional diagnostic accuracy when added to qualitative ^{18}F-FDG-PET/CT assessment.
3) ^{18}F-FDG-PET/CT should not be performed as a diagnostic tool within a month following surgery.

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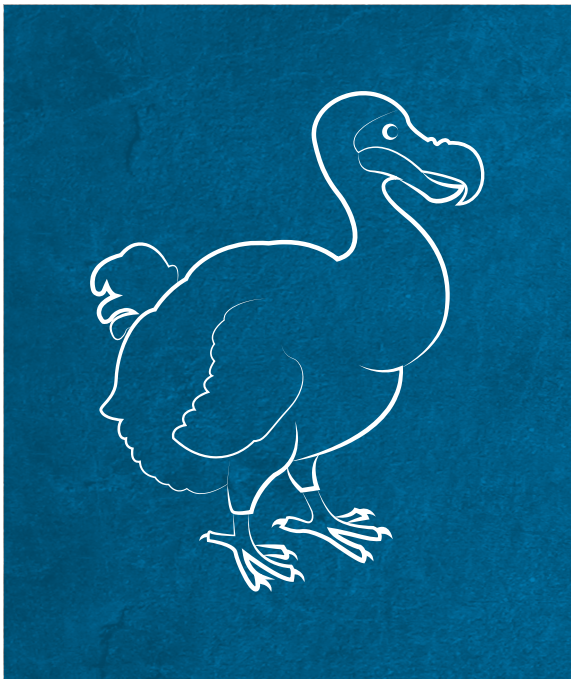

PART III

SERUM INFLAMMATORY MARKERS

CHAPTER 7

Limited predictive value of serum inflammatory markers for diagnosing fracture-related infections: results of a large retrospective multicenter cohort study

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ABSTRACT

Introduction. Diagnosing Fracture-Related Infections (FRI) based on clinical symptoms alone can be challenging and additional diagnostic tools such as serum inflammatory markers are often utilized. The aims of this study were 1) to determine the individual diagnostic performance of three commonly used serum inflammatory markers: C-Reactive Protein (CRP), Leukocyte Count (LC) and Erythrocyte Sedimentation Rate (ESR), and 2) to determine the diagnostic performance of a combination of these markers, and the additional value of including clinical parameters predictive of FRI.

Methods. This cohort study included patients who presented with a suspected FRI at two participating level I academic trauma centers between February 1st 2009 and December 31st 2017. The parameters CRP, LC and ESR, determined at diagnostic work-up of the suspected FRI, were retrieved from hospital records. The gold standard for diagnosing or ruling out FRI was defined as: positive microbiology results of surgically obtained tissue samples, or absence of FRI at a clinical follow-up of at least six months. The diagnostic accuracy of the individual serum inflammatory markers was assessed. Analyses were done with both dichotomized values using hospital thresholds as well as with continuous values. Multivariable logistic regression analyses were performed to obtain the discriminative performance (Area Under the Receiver Operating Characteristic, AUROC) of (1) the combined inflammatory markers, and (2) the added value of these markers to clinical parameters.

Results. A total of 168 patients met the inclusion criteria and were included for analysis. CRP had a 38% sensitivity, 34% specificity, 42% positive predictive value (PPV) and 78% negative predictive value (NPV). For LC this was 39%, 74%, 46% and 67% and for ESR 62%, 64%, 45% and 76% respectively. The diagnostic accuracy was 52%, 61% and 80% respectively. The AUROC was 0.64 for CRP, 0.60 for LC and 0.58 for ESR. The AUROC of the combined inflammatory markers was 0.63. Serum inflammatory markers combined with clinical parameters resulted in AUROC of 0.66 as opposed to 0.62 for clinical parameters alone.

Conclusion. The added value of CRP, LC and ESR for diagnosing FRI is limited. Clinicians should be cautious when interpreting the results of these tests in patients with suspected FRI.

INTRODUCTION

Fracture-Related Infection (FRI) is a challenging complication after surgical fracture treatment [1, 2]. Consequences include reoperations, prolonged treatment with antibiotics, prolonged immobilization, inability to participate in social and work-related activities, increased medical costs, loss of function and even amputation [3-5]. As with most medical conditions, a successful treatment outcome starts with an accurate diagnosis. The fact that the clinical presentation of infection can be obscured by apparently normal wound healing is one of the difficulties of diagnosing FRI. When wound healing is compromised, and the classical infection symptoms such as pain, increased temperature, local erythema and swelling are present, FRI is usually easy to recognize. However, FRI can also present less apparent with symptoms mimicking those of delayed- or non-union, such as pain, implant failure and impaired fracture healing. It might even be present without any clinical signs and symptoms at all [1, 6, 7].

Another difficulty has been that until recently, the literature regarding the diagnosis and treatment of FRI was hampered by the lack of a clear definition [4]. However, in 2017, the characteristics of a FRI were clearly defined in a consensus meeting between experts in the field of bone infection in collaboration with the Arbeitsgemeinschaft für Osteosynthesefragen (AO Foundation) and the European Bone and Joint Infection Society (EBJIS) [2]. Two levels of certainty around diagnostic features were defined. Signs that are suggestive of FRI can be clinical signs of infection (such as redness, fever and new onset of joint effusion), radiological signs (for example bone lysis, sequestration, implant loosening, nonunion and periosteal bone formation), wound drainage and elevated serum inflammatory markers. Confirmatory clinical signs are a fistula, sinus, purulent drainage or wound breakdown which communicates to the bone itself or to the fixation device. In absence of these confirmatory clinical signs, the diagnosis can be confirmed by either microbiology (with phenotypically indistinguishable pathogens identified by culture from at least two separate deep tissue/implant specimens) or histology (presence of microorganisms in deep tissue taken during an operative intervention) [2].

Elevated serum inflammatory markers are often used as diagnostic parameters for postoperative infections after orthopedic trauma surgery and are mainly investigated in PJIs [8, 9]. Although they are considered to be indicative for the presence of FRI according to the aforementioned consensus meeting, research focusing on the added value of these parameters for diagnosing FRI is limited [10-13]. In a recent survey amongst medical specialists involved in the care for patients with FRI, C-reactive protein (CRP) was regarded to be the most valuable tool for diagnosing FRI, followed by the Erythrocyte

Sedimentation Rate (ESR) and Leucocyte Count (LC) respectively [14]. However, the added value of serum inflammatory markers is still under debate. Large cohort studies which tell us whether these markers are capable of distinguishing a bacterial infection from a normal inflammatory response due to the injury, tissue damage, fracture healing, or the fracture surgery, are lacking so far [15-19]. It is therefore mandatory to assess the role of these serum inflammatory markers in the decision-making process for diagnosing FRI.

The two aims of the current study were:

1) To determine the individual diagnostic performance of the three commonly used serum inflammatory markers, CRP, LC and ESR, in FRI.
2) To assess the diagnostic value of a combination of these markers, and their value in addition to clinical parameters predictive of FRI.

PATIENTS AND METHODS

Study design

This is a retrospective cohort study performed at the University Medical Center Utrecht (UMCU) and the University Medical Center Groningen (UMCG), two Level I academic trauma centers in the Netherlands.

In- and exclusion criteria

In order to be able to calculate the accuracy of serum inflammatory markers in both patients with and without FRI, patients from a previous assembled database on medical imaging for suspected FRI were included. This database comprised of all patients who underwent nuclear medical imaging for suspected FRI between February 1st 2009 and December 31st 2017 of the UMCU and UMCG. In accordance with clinical practice, where serum inflammatory markers are ordered when an infection is suspected, blood sampling had to be obtained within a range of seven days around the date an FRI was first considered (mostly at the outpatient department). Cases missing inflammatory markers or outcome data due to incomplete reporting were excluded from the analyses. In uncomplicated orthopedic- and trauma surgical cases, levels of CRP peak at the second postoperative day. In uneventful cases, the CRP returns to normal values between day two to twelve postoperatively [20-25]. Maximum values of LC are seen on day one to three postoperatively and decline to normal values between day four to six [26]. Values of ESR peak at day seven to eleven postoperatively and decrease gradually

until after week six [19]. Therefore, patients were excluded who underwent surgery in 14 days preceding testing for CRP, 7 days for LC and 6 weeks for ESR testing. In- and exclusion criteria are presented in Table 1.

Table 1. Inclusion and exclusion criteria.

Inclusion	Exclusion
1. Patients with a suspected Fracture-Related Infection.	1. Patients who underwent surgery in the fourteen days preceding collection of the blood sample for determining the serum inflammatory markers 2. Pathologic fractures 3. Prosthetic joint infection (PJI) 4. Haematogenous infection 5. Patients with (auto-)immune diseases 6. Patients with (pre-)malignancies 7. Concomitant use of corticosteroids 8. Evident other focus of infection 9. No reference standard available (representative cultures or at least six months follow-up)

Ethical approval

The study protocol was evaluated by the institutional review board (medical ethical research commission, METC) of the UMCU and found to be exempted from further approval requirements (METC-17-694).

Serum inflammatory markers

The index test comprised of CRP and LC. Analysis was done similarly in both participating centers. In the UMCU, blood was drawn into a 2.0 mL vacuum tube (BD Vacutainer; BD Medical Systems, Franklin Lakes, NJ, USA) containing K2-EDTA as an anticoagulant for blood cell analysis and a 4.0 mL vacuum tube Lithium-Heparin as an anticoagulant for CRP measurement.

The UMCG used standard 4.0 mL K2 EDTA and 4.5 mL Lithium-Heparin tubes. All blood samples were analyzed in the central diagnostic laboratories of the UMCU and UMCG (both with full ISO-15189 accreditation). C-reactive protein (CRP) was measured using a turbidimetric immunoassay on a DxAU 5811 automated chemistry analyzer (Beckman-Coulter, Brea, CA, USA). Similar analysis was done in the UMCG using a Roche CRPL3 analyzer with wide range assay (Roche, Mannheim, Germany). LC was measured using a Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). This analyzer uses spectrophotometry, electrical impedance and laser light scattering

(multi angle polarized scatter separation, (MAPPS)) to classify blood cells [27, 28]. In the UMCG, similar analysis was done using a Sysmex XN-20 Automated hematology analyzer (Sysmex, Kobe, Japan). The validity of all test results was checked with built-in quality flags, daily quality control samples and external quality assessment schemes. The ESR was measured using a method according to Westergren. The UMCU uses whole blood anticoagulated with sodium citrate 3,2% (4:1) in combination with a ESR analyzer (Monitor V100, Vital Diagnostics, SrL, Forli, Italy), in the UMCG the ESR was measured in EDTA whole blood in diluted with sodium citrate 3,2% (4:1) combination with the Starrsed interliner (Mechatronics, Zwaag, the Netherlands) [29].

Although analyses of blood samples were done in a similar set-up, both participating centers used slightly different threshold values for the serum makers. Since statistical calculations in this paper were performed on data from both centers to improve the possible predictive performance, common threshold values used in clinical practice and reported in medical literature were used to reflect the current performance of the separate parameters. The threshold in this study for CRP was less than 5.0 mg/L and leukocyte count less than 10.0 x 10^9/L. For ESR, the threshold for men was 11 mm/h and for women 24 mm/h.

Clinical parameters

The clinical parameters included in the multivariate analysis were Gustilo-Anderson classification, ISS, diabetes mellitus, smoking status and lower extremity fractures. These parameters were used as these are known to increase the risk of a FRI [30].

Reference standard

The gold standard in the final diagnosis of FRI was based on the outcome of medical microbiology (MMB) results of at least two separate samples of deep tissue taken during a surgical intervention [2]. Two experienced trauma surgeons (GG and FIJ, >5 years board certified) assessed the validity of the MMB results. Only if two or more deep samples were taken from the suspected area of bone infection, the MMB results were regarded as relevant. Only when two or more samples were positive with both morphologically the same organism, the MMB results were regarded as positive. In case of no surgery (and therefore no intra-operative cultures), the definite diagnosis was based on a clinical follow-up of at least six months. Throughout the follow-up, a final diagnosis was made on basis of positive clinical confirmatory criteria. When the aforementioned confirmatory signs were present perioperatively, the patient was also considered to be suffering from FRI [2].

Data collection

The electronic patient files of all included patients were scrutinized on when an infectious complication was first suspected and data was collected on demographics, type of fracture according to the Müller AO Classification of Fractures [31], Gustilo Anderson classification in case of an open fracture [32], date, trauma mechanism, fracture type and surgical management of the index trauma, laboratory findings, microbiology results, final diagnosis and clinical outcome during follow-up.

Statistical analysis

Continuous data are presented as mean and standard deviation (SD) in case of normal distributions or median and interquartile range (IQR) when not normally distributed. The baseline characteristics per center were compared to analyze whether there were any substantial differences between the centers. Hypothesis testing was done using independent t-test or Mann-Whitney U test for the continues values, and Chi-squared test or Fisher's exact test for the dichotomized values. A p-value of <0.05 was considered significant.

In the first analysis, the serum markers were dichotomized using the aforementioned threshold values, as this reflects the diagnostic performance in current clinical practice. For each parameter, true positive (TP), true negative (TN), false positive (FP) and false negative (FN) results were described. Contingency tables were constructed. Sensitivity and specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratio's (LR+ and LR) were calculated. Second, to assess the maximal predictive performance, separate continuous values were used.

Third, to assess the diagnostic performance of the combination of the inflammatory markers, a multivariable logistic regression model including the inflammatory markers was fitted. Subsequently, two models were fitted to determine the added value of the inflammatory markers to the clinical parameters. The first one included the clinically predetermined parameters. The second one included these parameters, and also the combined inflammatory markers. To reduce the risk of overfitting, a maximum of one predictor per 5-10 events was used.

The diagnostic performance of these continuous models was assessed using the AUROC as a measure of discrimination. The Q-point method, which determines the threshold value closest to the upper left corner of the AUROC, was used to deduct the optimal threshold, for which the sensitivity and specificity were calculated.

Sensitivity analyses were performed to (1) assess whether the diagnostic performance of the multivariable logistic regression analysis differs per center, (2) whether the time interval (<14 days versus ≥14 days between inflammatory markers and intra-operative cultures) affects the diagnostic performance and (3) to assess whether the linearity assumption of the combined markers with the (logit) outcome affects the performance, through log-transforming the variables.

All data analyses were performed using the Statistical Package for Social Sciences (SPSS®) statistics for Windows (version 20.0.0.0, IBM, Armonk, NY, USA). Where applicable, the reporting of this study followed the Transparent Reporting of a multivariable Prediction Model for individual diagnosis or prognosis (TRIPOD statement) [33].

RESULTS

The cohort consisted of 365 patients who underwent medical imaging for suspected FRI. A total of 197 patients were excluded from analyses due to missing data on serum inflammatory markers (n=171) or other parameters. After exclusion, a total of 168 patients were included in this study. Basic demographics and clinical characteristics of the included patients from both participating centers are shown in Table 2. The cohort consisted predominantly of male patients (n=115, 68.5%) with a median age of 54 years (IQR 40-62). Fractures were most commonly located in the lower extremity (n=140, 83.4%). The study population consisted of patients who were suspected to suffer from long standing FRI. The median interval between initial fracture surgery and nuclear imaging for a suspected FRI was 480 (IQR 229-1312) days.

FRI in study population

Overall, FRI was present in 61 patients (36%). In the cohort, 41 patients were diagnosed with FRI on basis of MMB results. Twenty patients with negative or without MMB results developed FRI during the follow up. The median clinical follow up in the cohort was 53 (IQR 45-134) weeks. Median interval between blood sampling for laboratory analysis and operatively obtained samples for MMB was 49 (IQR 19-85) days.

Table 2. Baseline characteristics of study population.

	Both centers	UMCU (n=41)	UMCG (n=127)	*p*-value
Age (median (IQR))	54 (40-64)	58 (47-63)	54 (38-64)	0.27
Age at onset (median (IQR))	51 (36-59)	53 (45-59)	51 (36-62)	0.26
Sex				
Male	115 (68.5%)	26 (63.4%)	89 (70.1%)	0.44
Comorbidities				
Diabetes mellitus	13 (7.7%)	5 (12.2%)	8 (6.3%)	0.31
Psychiatric disorder	11 (6.5)	2 (4.9%)	9 (7.1%)	0.47
Obesity	21 (12.5%)	2 (4.9%)	19 (15.0%)	0.11
Osteoporisis	5 (3.0%)	5 (12.2%)	0 (0%)	0.35
Hypothyroidism	3 (1.8%)	1 (2.4%)	2 (1.6%)	0.57
Risk factors				
Smoking	63 (37.5%)	14 (34.1%)	49 (38.6%)	0.71
NSAIDs	31 (18.5%)	5 (12.2%)	26 (20.5%)	0.26
Soft drugs	6 (3.6%)	2 (4.9%)	4 (3.1%)	0.64
Hard drugs	6 (3.6%)	2 (4.9%)	4 (3.1%)	0.64
Alcoholabuse	7 (4.2%)	2 (4.9%)	5 (3.9%)	0.68
ASA classification				0.40
I	58 (35.5%)	14 (34.1%)	44 (39.3%)	
II	72 (42.9%)	20 (48.8%)	52 (46.4%)	
III	20 (11.9%)	4 (9.8%)	16 (14.3%)	
IV	1 (0.6%)	1 (2.4%)	0 (0.0%)	
Unknown	17 (10.1%)	2 (4.9%)	15 (11.8%)	
BMI, n = 150 (mean (SD)) Unknown (n=)	28,18 (5.38) 18 (10.7%)	26.91 (4.68) 1 (2.4%)	28,77 (5.54) 17 (13.4%)	0.06
ISS				**<0.001**
<16	114 (67.9%)	16 (39.0%)	99 (78.0%)	
>16	39 (23.2%)	18 (43.9%)	21 (16.5%)	
Unknown	15 (8.9%)	7 (17.1%)	7 (5.5%)	
Fracture location				**0.002**
Upper extremity	18 (10.7%)	1 (2.4%)	17 (13.4%)	
Lower extremity	140 (83.3%)	33 (80.5%)	107 (84.3%)	
Spine	7 (4.2%)	5 (12.2%)	2 (1.6%)	
Pelvis	3 (1.8%)	2 (4.9%)	1 (0.8%)	
Fracture type				0.85
Open	80 (47,6%)	18 (43.9%)	62 (48.8%)	
Closed	79 (47.0%)	16 (39.0%)	63 (49.6%)	
Unknown	9 (5.4%)	7 (17.1%)	2 (1.6%)	
Gustilo-Anderson Classification (32)				**0.04**
Grade 1	16 (9.5%)	3 (7.3%)	13 (10.2%)	
Grade 2	12 (7.1%)	0 (0.0%)	12 (9.4%)	
Grade 3	43 (19.7%)	11 (26.8%)	22 (17.4%)	
Unknown	19 (11.3%)	4 (9.8%)	15 (11.8%)	

Diagnostic performance of serum inflammatory markers

Details on the serum markers are shown in Table 3. For CRP, there were 49 TP, 36 TN, 69 FP and 10 FN results. This corresponds to 83% sensitivity and 34% specificity. When considering CRP as a continuous variable, an AUROC of 0.64 (0.55-0.72) was found. The optimum threshold was 10.5 mg/L, with a corresponding 61.0% sensitivity and 62.9% specificity. For leukocyte count, there were 22 TP, 72 TN, 26 FP and 35 FN results. This resulted in a 39% sensitivity and 74% specificity. When analyzed as a continuous variable the AUROC was 0.60 (0.50-0.69). The optimum threshold was 8.6 $x10^9$/L, with a corresponding 60.0% sensitivity and 61.2% specificity. Regarding ESR, there were 18 TP, 35 TN, 11 FP and 22 FN results. This is consistent with 45% sensitivity and 76% specificity. When analyzed as a continuous variable, the AUROC was 0.58 (0.46-0.71). At the optimum threshold (10.0), sensitivity was 72.4% specificity 50.1%. The results are presented in Table 4 and Table 5.

Table 3. CRP, LC and ESR.

	FRI				No FRI			
	TP	**TN**	**Median**	**IQR**	**FP**	**FN**	**Median**	**IQR**
CRP	49	36	15.0 mg/L	5.0-60.0 mg/L	69	10	7.0 mg/L	4.1-18.5 mg/L
LC	22	72	9.3 $x10^9$/L	7.1-12.4 $x10^9$/L	26	35	8.1 $x10^9$/L	6.7-10.2 $x10^9$/L
ESR	18	35	18.0 mm/h	7.0-36.0 mm/h	11	22	11.0 mm/h	5-31.5 mm/h

Table 4. Diagnostic accuracies for CRP, LC and ESR.

Test	**CRP**	**LC**	**ESR**
Sensitivity (95% CI)	83.1% (71.0%-91.6%)	38.6% (22.0%-52.4%)	45.0% (29.3% - 61.5%)
Specificity (95% CI)	34.3% (25.3%-44.2%)	73.5% (63.6%-81.9%)	76.1% (61.2% - 87.4%)
PPV (95% CI)	41.5% (37.2%-46.0%)	45.8% (34.7%-57.4%)	62.1% (46.8% - 75.2%)
NPV (95% CI)	78.3% (65.9%-87.0%)	67.3% (61.9%-72.3%)	61.4% (53.5% - 68.7%)
LR+ (95% CI)	1.26 (1.06-1.51)	1.45 (0.91-2.31)	1.88 (1.01 - 3.49)
LR- (95% CI)	0.49 (0.26-0.92)	0.84 (0.66-1.06)	0.72 (0.52 - 1.00)
Accuracy	51.8% (43.9%-59.7%)	60.7% (52.5%-68.4%)	79.6% (64.7% - 90.2%)

Table 5. Diagnostic accuracies for continuous variables CRP, LC, ESR and CRP + LC.

Test	CRP	LC	ESR	CRP + LC
AUROC	0.64 (95% CI 0.55-0.72)	0.60 (95% CI 0.50-0.69)	0.58 (95% CI 0.46-0.71)	0.63 (95% CI 0.54-0.73)
Sensitivity	61.0%	60.0%	72.4%	60.0%
Specificity	62.9%	61.2%	50.1%	63.9%

Multivariable logistic regression analysis

ESR was left out of these analyses as this marker was missing in half of the patients (n=86, 51.2%). The AUROC of CRP and LC combined was 0.63 (95% CI 0.54-0.73). At the Q-point, there were 33 TP, 62 TN, 35 FP and 22 FN, with a sensitivity and specificity of 60% and 64% (Table 4 and Table 5). The model with clinical parameters and combined inflammatory markers had an AUROC of 0.66 (95% CI 0.55-0.77), as compared to 0.62 (95% CI 0.51-0.72) without inflammatory markers.

The AUROC of the combined markers per center was 0.63 (0.54-0.73) for the UMCG, and 0.68 (0.51-0.87) for the UMCU. The AUROC was 0.64 (0.34-0.93) <14 days and 0.61 (0.48-0.75) ≥14 days. The AUROC of the model with log-transformed CRP and LC was 0.63 (0.54-0.73).

DISCUSSION

This study focused on the diagnostic accuracy of the serum inflammatory markers CRP, LC and ESR in patients who were suspected of FRI. It is the first study to include clinical parameters proven to be predictive of FRI in its analysis. Although most clinicians regard serum inflammatory markers to be part of the general work-up of suspected FRI, the results of this study indicate that they should be cautious when interpreting their results, as was published in the Consensus definition on FRI [2].

The majority of the literature on inflammatory markers in orthopedic infection has focused on periprosthetic joint infections (PJI) and osteomyelitis of the diabetic foot [34-37]. CRP has been proven to be useful in both [38, 39]. Moreover, the value of LC is less well established [9, 40]. In early postoperative infections after fracture surgery, continuous elevation or a secondary rise might be expected in CRP and LC [24, 41]. Levels of serum CRP, LC and ESR have been shown to be significantly lower in FRI than in haematogenous osteomyelitis and osteomyelitis of the diabetic foot [42, 43].

Studies on the diagnostic value of serum inflammatory markers in FRI are limited, and their methodology is heterogeneous. Different serum marker thresholds are used, and study populations vary. As in the current study, the study population of Buhl et al. consisted of patients who underwent nuclear medical imaging for suspected FRI or infected prosthesis [44]. They reported a sensitivity and specificity for ESR of 84% and 29% respectively, and 56% and 35% for CRP. These results differ from those in the current study. This may be due to PJI being excluded in the current study and the use of different thresholds. Most studies on serum markers in FRI have focused on subgroups of FRI, such as infected non-union or patients undergoing conversion surgery. One study reported on the value of CRP and ESR in diagnosing infection in patients undergoing conversion from internal fixation of a femoral neck fracture to total hip arthroplasty [45]. The authors reported a higher diagnostic accuracy than the current study, with an AUROC of 0.89 for both markers. Unfortunately, their study has a high risk of overfitting due to the inclusion of only six patients with FRI. Therefore, the true AUROC, obtained after (internal and) external validation, will be much lower [46]. Several studies have focused on the value of inflammatory markers in diagnosing infection in patients presenting with mal- or non-union [11-13]. The diagnostic accuracy of individual serum inflammatory markers in this sub-group of FRI is low. Some of these studies have looked at the diagnostic accuracy of combined serum markers. Similar to the results of the current study, combining markers was found to increase the diagnostic accuracy for FRI only marginally [11, 13].

With an accuracy of 79.6%, the diagnostic value of ESR in the current study appears to be high. However, the large overlap in the IQR of the FRI and non-FRI groups shows the discriminative value of ESR to be low.

The differences in results between the literature and the current study may be caused by several factors. Most importantly, several different thresholds are used to define elevation of serum inflammatory markers. This makes a valid comparison of results impossible, especially when only sensitivity and specificity are reported. Furthermore, FRI is a heterogeneous disease, with tissue involvement varying in location and severity. Some studies focus on all patients with FRI, others choose subgroups to increase population homogeneity. These differences in study populations further complicate comparing results and it is therefore imperative that international lab protocols are being developed and uniform diagnostic criteria including threshold values and timing for obtaining serum inflammatory markers regarding FRI are being established and implemented. Finally, most studies have looked at serum markers taken between 1 to 14 days prior to obtaining intra-operative cultures. The current study focused on inflammatory markers when infection was first suspected, with a median of 48.5 days

between index- and reference test. This is in concordance with clinical practice, as the clinician will obtain serum inflammatory markers at the time an FRI has to be confirmed or ruled out. The actual surgery often follows at a later point, when additional diagnostic work, such as imaging, has been completed. This difference may have influenced the results.

Strengths of this study are that it is one of the largest cohorts investigating the diagnostic performance of individual and combined serum inflammatory markers in FRI. The inclusion of combined markers is important, as in clinical practice, inflammatory markers are never interpreted individually. Furthermore, they are always interpreted in combination with clinical parameters. Therefore, information from multiple markers was combined with clinical parameters that are associated with FRI to estimate the probability of infection.

This study does have some limitations. First of all, all patients with suspected FRI were collectively analyzed, and thus these results may not be applicable to all possible subgroups. Furthermore, due to its retrospective nature, there was no uniform time interval between index- and reference test. However, this is in accordance with clinical practice. In addition, the laboratory measurements have been performed using different methods, however due to laboratory standardization and internal and external quality control schemes differences due to measurement methods are negligible. Also, the outcome of this study might be affected by selection bias as the patients undergoing advanced nuclear imaging could have been selected based on the outcome of their serum inflammatory marker testing. This could potentially alter the true NPV of the markers.

CONCLUSION

The outcome of this retrospective study indicates that the added diagnostic value of CRP, LC and ESR seems to be limited for FRI. FRI can still be present when serum inflammatory markers are within normal range. Therefore, clinicians should be cautious when interpreting the results of these tests in patients with suspected FRI.

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CHAPTER 8

Diagnostic accuracy of serum inflammatory markers in late fracture-related infection: a systematic review and meta-analysis

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ABSTRACT

Aims. To assess the diagnostic value of C-reactive protein (CRP), leukocyte count (LC) and erythrocyte sedimentation rate (ESR) in late fracture related infection (FRI).

Methods. PubMed, Embase and Cochrane databases were searched focusing on the diagnostic value of CRP, LC and ESR in late FRI. Sensitivity and specificity combinations were extracted per marker. Average estimates were obtained using bivariate mixed effects models.

Results. A total of 8280 articles were identified, six were included. Sensitivity of CRP ranged from 60.0-100.0% and specificity from 34.3-85.7% in all articles. Five articles were pooled, showing a sensitivity and specificity of 77.0% and 67.9% respectively. For LC, this was 22.9-72.6%, and 73.5-85.7% respectively in five articles. Four articles were pooled, resulting in a 51.7% sensitivity and 67.1% specificity. For ESR, sensitivity and specificity ranged from 37.1-100.0% and 59.0-85.0% respectively in five articles. Three articles were pooled, showing a 45.1% sensitivity and 79.3% specificity. Four articles analyzed the value of combined inflammatory markers, reporting an increased diagnostic accuracy. These results could not be pooled due to heterogeneity.

Conclusion. The serum inflammatory markers CRP, LC and ESR are insufficiently accurate to diagnose late FRI, but they may be used as a suggestive sign in its diagnosis.

INTRODUCTION

Fracture-Related Infection (FRI) is a challenging complication in orthopaedic trauma surgery and uncertainties exist in both diagnostic and treatment strategies [1]. Regardless of antibiotic prophylaxis and sterile precautions in the operation room, the incidence of infection after fracture treatment is relatively high, generally varying between 1 and 30% depending on comorbidities, fracture type and soft tissue injury [2-5]. FRIs often result in multiple re-operations, long antibiotic treatment, immobilization and restrictions to participate in work and social activities [6-9].

Although classical clinical signs typically seen in infection (such as redness, swelling, pain and warmth) are often more prominent in early compared to late cases, symptoms can be subtle in both groups and may be relapsing and remitting over long periods of time [10]. Several additional diagnostic modalities have currently been proposed, such as medical imaging [11] and histological testing [12]. According to the 2017 consensus definition, criteria to establish the presence or absence of FRI are *confirmatory* (infection definitely present) or *suggestive* (infection possibly present) [13]. Suggestive diagnostic criteria include elevated C-reactive protein (CRP), leukocyte count (LC) and/or erythrocyte sedimentation rate (ESR). Although these markers are part of the consensus definition for FRI and commonly used as a diagnostic and severity parameter for postoperative infections after orthopaedic trauma surgery, its accuracy has mainly been investigated in prosthetic joint infections (PJI) and patients with diabetic foot osteomyelitis [14-19].

Generally, raised inflammatory markers are considered to be suggestive of infection in case of secondary rise after initial decrease, or when a consistent elevation is present over a long period of time [13]. In FRI, elevations in inflammatory markers may be more subtle compared to PJI or diabetic foot osteomyelitis [20]. In addition, an elevation in these markers may be seen in trauma patients due to systemic inflammatory response, post-operative or post-trauma tissue damage or other, non-surgical infections during the postoperative period [21-24]. It is this clinical variation, together with limited evidence in the literature, that makes the exact role of serum inflammatory markers as part of the diagnostic algorithm for FRI unclear.

The aim of this study was to assess the diagnostic value of CRP, LC and ESR in late fracture-related infection.

MATERIALS AND METHODS

Search strategy

On March 26th 2018, a computer aided systematic literature search was performed in the PubMed, EMBASE and Cochrane libraries. Articles in the English, Dutch and German language were included. Considering the time interval there were no limitations. Search terms were defined by the authors and reviewed by a professional information retrieval specialist. The search strings are available in Appendix 1. Appendix 1 svp na de references. Articles were first screened on title and abstract. Two reviewers (JK and PB) scored all articles independently. A third reviewer was consulted in case of indecision to assess whether the articles met the inclusion criteria (GG). Subsequently, the full-text of the included articles was reviewed by all three reviewers. In addition, cross-reference checking of included articles and of relevant review articles was performed.

Study selection

This review focuses on the diagnostic accuracy of the most commonly utilized serum inflammatory markers for detecting late FRI, namely CRP, LC and ESR, individually or combined. Therefore, information on other diagnostic inflammatory markers was disregarded. Articles solely reporting on early FRI (onset less than six weeks after the operation [10] were excluded as 1) early FRI usually poses a less complex diagnostic dilemma and 2) it was felt by the authors that early and late infections are different entities and should be analysed separately to prevent confounding bias. Both patients with or without fracture fixation implants in situ were eligible for inclusion. Articles solely reporting on other types of bone or non-trauma related infections such as PJI, diabetic feet, spondylodiscitis and haematogenous osteomyelitis were excluded. Furthermore, articles without a solid reference test (defined as intra-operative cultures or clinical follow up of at least five months) for confirmation of the infection were excluded. Papers reporting on the results of a heterogeneous patient population were included, as long as separate analyses for FRI were provided. This is specifically stated in the results section if applicable. No concessions were made for non-trauma-related articles. The inclusion and exclusion criteria are presented in Table 1.

Data collection and extraction

From all included articles, the following data was extracted: 1) author; 2) year of publication; 3) study type and population; 4) number of patients included; 5) results of index test; 6) results of reference test (the gold standard); 7) diagnostic accuracy (any

measures) of the serum inflammatory markers for late FRI. Data was extracted by two reviewers independently (JK and PB). All authors were contacted when raw data were not reported in the articles.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
1. The study must analyse serum inflammatory parameters C-reactive protein (CRP), leukocyte count (LC) (or: white blood cell count) and erythrocyte sedimentation rate (ESR).
2. The study must evaluate late fracture related infection (or a synonym), defined as onset later than six weeks after surgical intervention.
4. A valid reference test must be used in the study defined as intra-operative cultures or clinical follow up of at least five months.
5. The study must provide a clear analysis of the investigated serum inflammatory parameters in order to construct contingency tables of relevant results.
6. The study must be conducted on humans.
Exclusion criteria
1. Articles that investigate forms of non-traumatic osteomyelitis, such as acute osteomyelitis and osteomyelitis due to peri-prosthetic infections, diabetic feet and haematogenous infections. 2. Articles that consist of less than 5 participants.
3. Articles not written in the English, Dutch or German language.
4. Poster/conference papers.

Methodological quality assessment

Assessment of risk of bias and applicability of the study design of the included articles was performed using the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Articles, version 2). The QUADAS-2 tool consists of four domains: patient selection, index test, reference standard and flow and timing [25]. The methodological quality of the articles was assessed by two reviewers independently (JK and PB). A third reviewer confirmed the outcomes of the QUADAS-2 tool for the included articles (GG). Since one selected study [26] was (co-)authored by the same authors as the current review, its methodological quality was assessed by an independent author (WJM). Authors were contacted when information regarding the quality of the study was not provided in the articles.

Statistical analysis

To assess the diagnostic performance per study, first the sensitivity and specificity were calculated from the (reconstructed) 2x2 contingency tables from the included articles. These were graphically visualized in a forest plot, along with their confidence interval. The individual sensitivities and specificities in summary measurement were not

directly pooled, because the included articles are likely to have used different (explicit or implicit) threshold values. Explicitly, researchers often use the threshold which is in use at their institution and these thresholds often differ between institutions. Implicitly, there could be variations in the thresholds (even if they are explicitly the same) due to differences in observers, laboratory protocols or equipment. These threshold values are a problem in obtaining pooled estimates of sensitivity and specificity as the natural trade-off between sensitivity and specificity means that a lower used threshold for an inflammatory marker leads to a higher sensitivity but lower specificity for FRI, and vice versa [27].

The reported pairs of sensitivity and specificity were graphically visualized. These plots were used to assess heterogeneity in discriminative performances between the articles. If the amount of clinical and statistical heterogeneity was considered acceptable, a summary measurement and expected Receiver Operating Characteristic (ROC) curve of the sensitivities and specificities was obtained. This was done while accounting for the (explicitly and implicitly) different thresholds, using a bivariate mixed effects model [27, 28]. This model first jointly incorporates both the degree of inter- and intra-study variation in sensitivity and specificity to calculate the corresponding confidence intervals per study. Second, these parameters were combined to obtain the summary ROC curve as a measure of the average discriminative performance. Summary ROC plots were obtained for both the separate and the combined inflammatory markers.

All analyses were performed using R software for statistical computing version 3.3.2 [29] with the additional package "mada" [30] and "forestplot" [31]. This systematic review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [32] and its "Explanation and Elaboration" [33].

RESULTS

Included articles

The search flow diagram is displayed in Figure 1. A total of 9860 articles met the search criteria. Additional data was provided by three authors (34-36). Ultimately, six articles remained for qualitative assessment [26, 34-38]. No articles were excluded after qualitative assessment, and all six articles were included in this systematic review, providing evidence on 582 patients. All included articles covered late FRI.

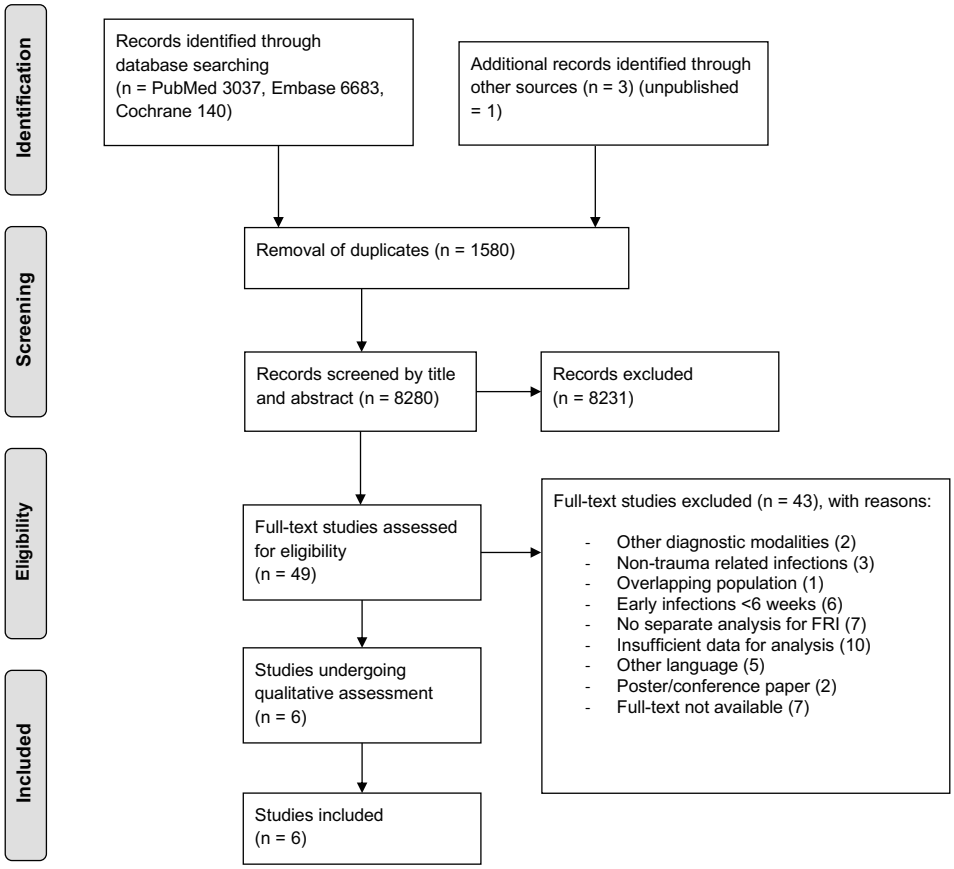


Figure 1. Flow Diagram (PRISMA).

Study quality

The results of the risk of bias and applicability assessment are presented in Figure 2. Concerns were mainly raised in regard to index- and reference test, and study flow and timing.

Study characteristics

The characteristics of the included studies are presented in Table 2. Four articles focused on the value of combining markers [26, 35, 37, 38].

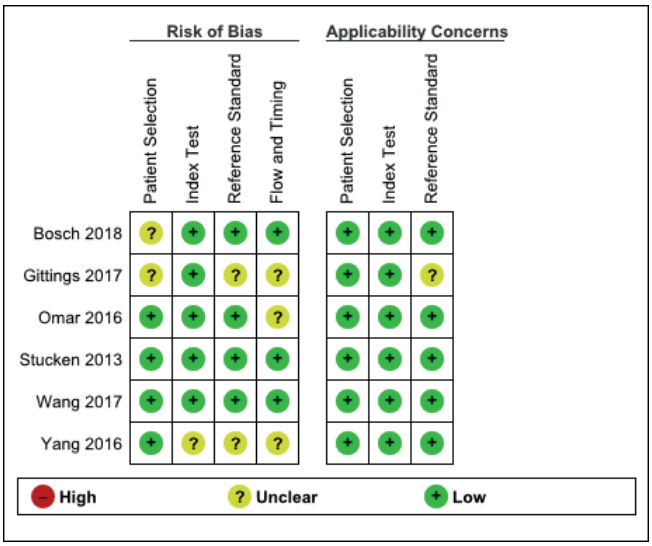


Figure 2. QUADAS-2 assessment for risk of bias and applicability.

C-reactive protein

All six included articles reported on CRP in their analysis. Three had populations consisting of patients with non-union [36-38], two focused on patients undergoing revision surgery after initial internal fixation [34, 35] and one investigated patients undergoing nuclear medical imaging for suspected FRI [26]. The results can be found in Table 2/Figure 3. Thresholds used to define elevation varied between 5.0-10.0 mg/L, and all articles used intra-operative cultures as a reference test. Overall, the sensitivity for detecting FRI varied between 60.0-100.0%, and specificity varied between 34.3-85.7%.

Leukocyte count

Five articles included LC in their analysis [26, 34, 36-38]. Three focused on patients presenting with un-united fractures [36-38]. The other two investigated patients undergoing revision surgery after initial internal fixation [34] and patients who underwent nuclear imaging for suspected FRI [26]. Thresholds used were comparable, ranging from 10.0-10.2 $x10^9$ cells/L, and all articles used intra-operative cultures as a reference test. Reported sensitivity varied between 22.9-72.6%, and specificity between 73.5-85.7%.

Table 2. Characteristics and results of included articles

Author	Year	Study type and population	Sample size (n)	FRI (n)	Reference test	Markers	Thresholds	Sensitivity	Specificity
Bosch et al.	2018	Retrospective cohort. Nuclear medical imaging for suspected FRI.	168	61	Intra-operative cultures with at least two sites revealing the same pathogen, presence of sinus tract or intra-operative purulence, or >6 months follow up.	CRP LC ESR	5 mg/L 10 x 10^9 cells/L 11 (m), 24 (f) mm/h	83.1% 38.6% 45.0%	34.3% 73.5% 76.1%
Gittings et al.	2017	Retrospective cohort. Conversion to total hip arthroplasty after initial internal fixation.	33	6	Intra-operative cultures or pre-operative diagnosis using MSIS criteria for PJI.	CRP ESR	7 mg/L 30 mm/h	100.0% 100.0%	81.0% 85.0%
Omar et al.	2016	Prospective cohort. Revision surgery after initial internal fixation.	62	51	Intra-operative cultures with at least two sites revealing the same pathogen, presence of sinus tract or intra-operative purulence.	CRP LC	5 mg/L 10.2 x 10^9 cells/L	78.4% 72.6%	72.7% 81.8%
Stucken et al.	2013	Prospective cohort. Non-united fractures.	93	30	Positive intra-operative cultures or gross infection at time of surgery or in the immediate post-operative period.	CRP LC ESR	10 mg/L 10 x 10^9 cells/L 30 mm/h	NE NE NE	NE NE NE
Wang et al.	2017	Retrospective cohort. Non-united fractures.	42	35	Intra-operative cultures with at least two sites revealing the same pathogen.	CRP LC ESR	8 mg/L 10 x 10^9 cells/L 20 mm/h	60.0% 22.9% 37.1%	85.7% 85.7% 71.4%
Yang et al.	2016	Retrospective cohort. Non-united fractures.	184	96	Intra-operative cultures, presence of a sinus tract, or purulence.	CRP LC ESR	8 mg/L 9.15 x 10^9 cells/L 15 (m), 20 (f) mm/h	68.8% 40.9% 74.2%	81.8% 79.4% 59.0%

CRP: C-reactive protein,LC: leukocyte count, ESR: erythrocyte sedimentation rate,MSIS: Musculoskeletal Infection Society,PJI: prosthetic joint infection, NE: Not estimable.

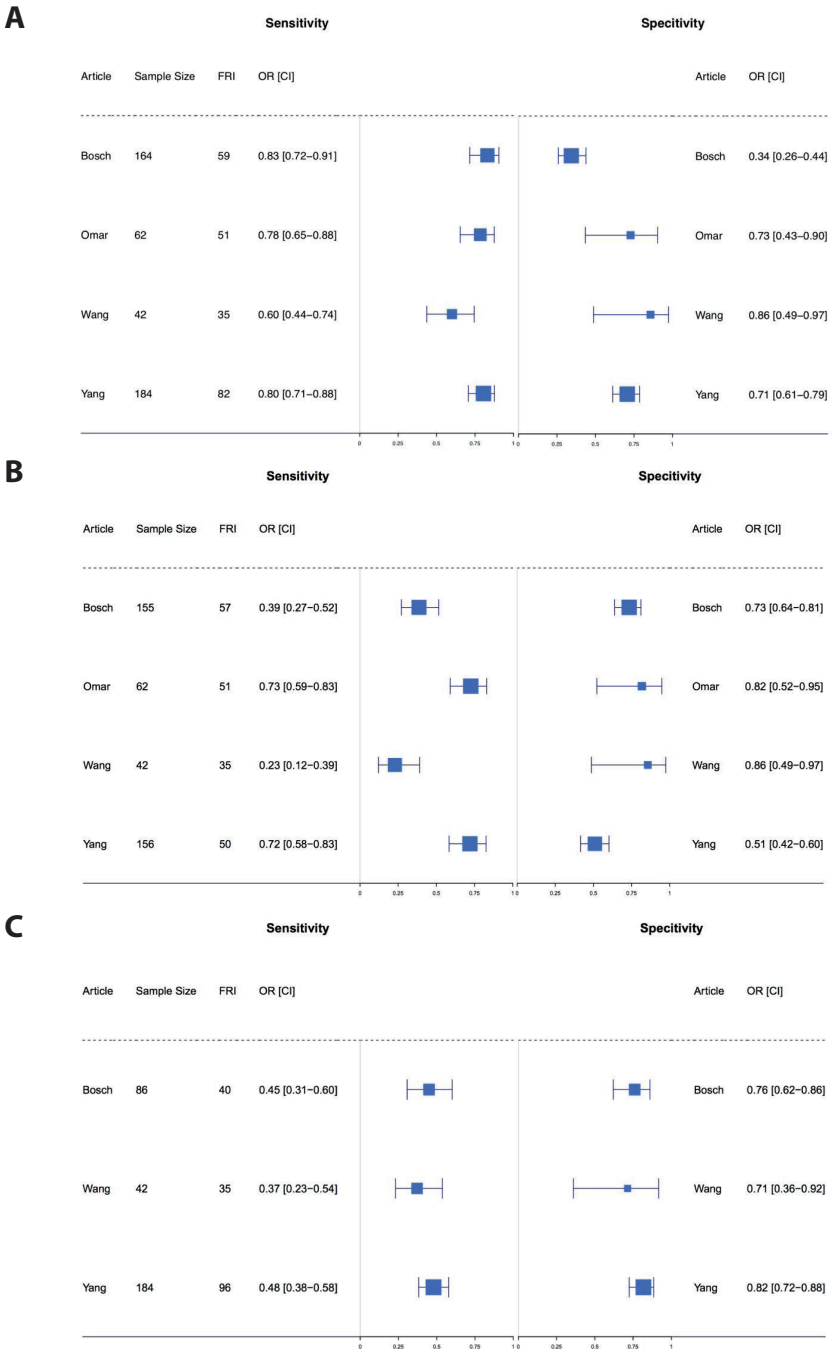


Figure 3 A-C. Forest plot sensitivity and specificity of markers CRP **(A)**, LC **(B)** and ESR **(C)** for diagnosing FRI.

Erythrocyte sedimentation rate

Five articles reported on ESR in their analysis [26, 35-38]. Three included ESR in their analysis on diagnosing infection in patients with un-united fractures [36-38], one studied the value of ESR in diagnosing infection in patients undergoing nuclear imaging for suspected FRI [26], and one focused on patients undergoing conversion to total hip arthroplasty after failed initial internal fixation [35]. Thresholds varied between 11.0-30.0 mm/h, with two articles using different threshold for men and women [26, 36]. All articles used intra-operative cultures as a reference test [37]. Overall, the reported sensitivity varied between 37.1-100.0%, and specificity varied between 59.0-85.0%.

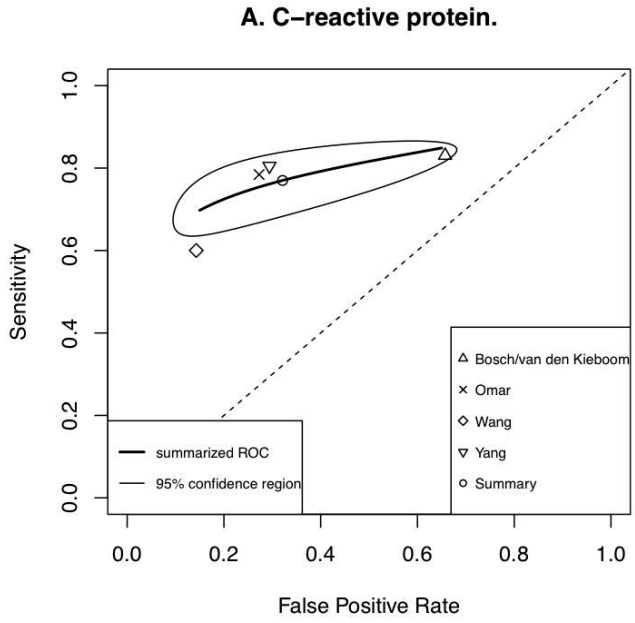
Combined scores

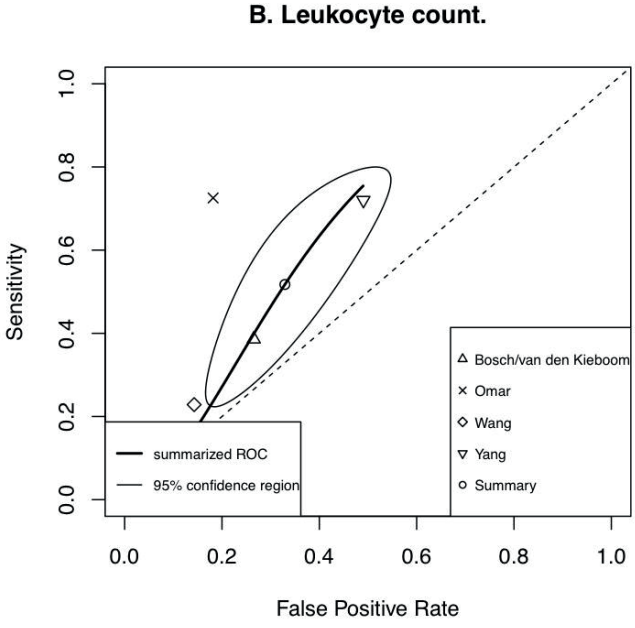
Four articles reported on the added value of combining markers [26, 35, 37, 39]. Two reported on combining up to four markers without specifying which markers [37, 38]. One study reported a predicted probability value of two and three combined positive tests [37]. They found a predicted probability of 56.0% when combining any two markers, and 100.0% when all three markers (CRP, LC and ESR) are elevated. Another study also reported on combining CRP, LC and ESR [38]. With any two markers combined, a predicted probability of 90.9% was calculated. When all three markers were elevated, a combined predicted probability of 100.0% was found. One study reported on the combination of CRP and ESR with a 83.0% sensitivity and 88.0% specificity [38]. One study reported on CRP and LC finding a 60.0% sensitivity and 64.0% specificity [26].

Meta-analysis

Articles were grouped per individual marker. Two by two contingency tables (true positive (TP), false negative (FN), false positive (FP), true negative (TN)) could be constructed from the pooled results of four articles for CRP (n=452) [26, 34, 36, 38], of four articles for LC (n=415) [26, 34, 36, 38], and of three articles for ESR (n=312) [26, 36, 38]. The sensitivities and specificities of the articles within the analysis of each serum marker showed acceptable comparability, and could therefore be pooled. This resulted in a sensitivity and specificity of 77.0% (95% CI 66.5-85.0%) and 67.9% (95% CI 38.7-87.6%) for CRP, 51.7% (95% CI 27.2-75.5%) and 67.1% (95% CI 19.3-50.2%) for LC, and 45.1% (95% CI 37.8-52.6%) and 79.3% (95% CI 71.7-85.2%) for ESR (Figure 4).

Due to heterogeneity, the articles reporting on combined markers could not be pooled (Figure 5).


A. C−reactive protein.
Sensitivity
False Positive Rate
0.0
0.2
0.4
0.6
0.8
1.0
summarized ROC
95% confidence region
Bosch/van den Kieboom
Omar
Wang
Yang
Summary


B. Leukocyte count.
Sensitivity
False Positive Rate
0.0
0.2
0.4
0.6
0.8
1.0
summarized ROC
95% confidence region
Bosch/van den Kieboom
Omar
Wang
Yang
Summary

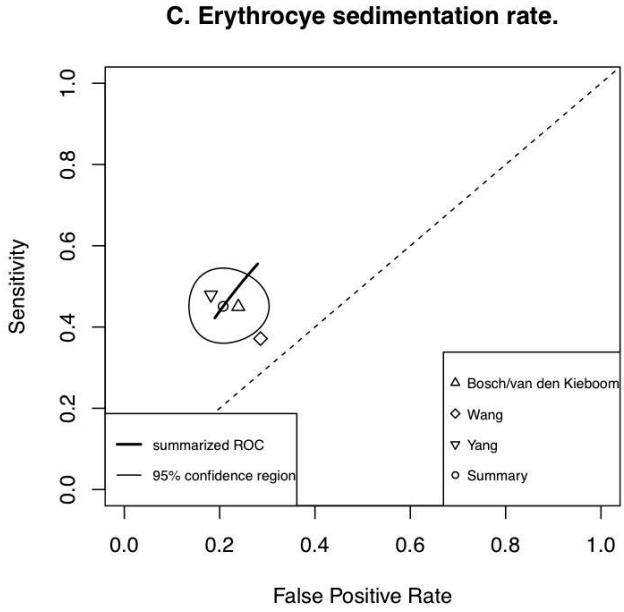


Figure 4 A-C. Summary ROC curves individual markers CRP **(A)**, LC **(B)** and ESR **(C).**

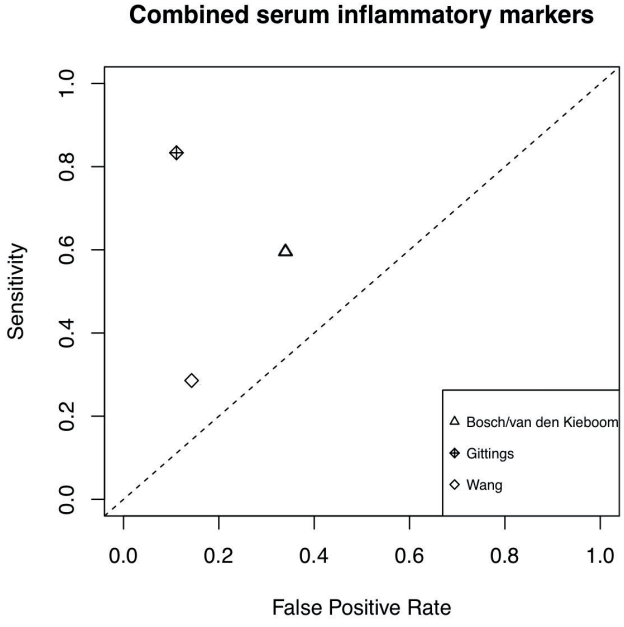


Figure 5. Summary ROC curve combined markers.

DISCUSSION

This review presents the current evidence on the diagnostic value of the serum inflammatory markers CRP, LC and ESR for late FRI. Meta-analysis of the pooled results showed limited diagnostic value of all three markers individually. Combined scores are shown to increase diagnostic performance, yet the accuracy remains insufficient in most articles.

Overall, the results of all markers vary greatly between the included articles. One of the difficulties that were encountered in this review was the fact that serum inflammatory markers were measured using different apparatus and methods. Also, the articles included in this review used several different thresholds when dichotomizing the serum inflammatory markers. The use of different thresholds complicates direct comparison of diagnostic performance between articles. Also, as these markers are measured on a continuous scale, dichotomization decreases their diagnostic potential. Therefore, articles on their diagnostic performance should (also) analyse these markers continuously in order to assess their potential and, subsequently, determine ideal threshold values. The value at which a sensitivity of >90% is reached, should serve as the threshold in suspected late FRI.

FRI encompasses a broad spectrum of manifestations, which can vary greatly in severity, location and duration. Study populations often consist of sub-groups of FRI, like infected non-union, patients undergoing revision surgery or certain types of medical imaging without specifying the pre-test probability. This results in heterogenic study populations, further complicating comparison of diagnostic performance between articles.

All of the included articles used intra-operative cultures as a reference test. However, there were variations in the specific culture methods used. Differences were seen in the number of samples taken, ranging from three to five. Some articles consider FRI to be present when the culture result of a single sample was positive [35, 37], while others require the same pathogen to be present in at least two different samples [26, 34, 38]. Also, details on collecting and culturing protocols were not always provided. Until the 2017 consensus meeting of experts in the field of bone infection, there was no uniform definition for FRI [13]. Only since then, agreement has been reached on a reference standard, and protocols for collecting intra-operative cultures have been formed [13, 39].

Since serum inflammatory markers are used in clinical practice to rule out FRI, a high sensitivity is needed. A high specificity is needed in order to prevent unnecessary invasive surgery and anti-microbial therapy in patients with a false positive diagnosis. Only one study found a sensitivity >90%. However, they included only six patients with FRI, increasing the risk of overfitting [35]. Specificity was generally low in all articles, increasing the risk of over-treatment if inflammatory markers are relied upon.

Although the results of this review show that dichotomized results of individual serum inflammatory markers have insufficient diagnostic performance, they may still be a suggestive sign of FRI. One way of increasing the diagnostic performance is by combining markers. This resembles clinical practice, where inflammatory markers are rarely interpreted on a standalone basis. Usually, multiple markers are interpreted in addition to clinical signs when estimating the likelihood of FRI. Only one study assessed the combination CRP, LC, ESR and clinical parameters predictive of FRI, and reported a limited added value of these inflammatory markers [26]. The other articles reported increased diagnostic performance when combining markers [35, 37, 38]. However, the diagnostic performance remains insufficient in most articles.

We recommend that international laboratory protocols for serum inflammatory markers become standardized in order to compare articles in a more reliable way and improve the diagnosis of late FRI in a clinical setting. Furthermore, uniform definitions and diagnostic criteria, as recently published in the consensus definition [13], should be implemented in both clinical practice and research.

This review has some limitations. Most articles on this topic suffer from small and heterogeneous patient populations, underreporting regarding laboratory techniques, different thresholds used and lack of a reference standard. Therefore, only six articles could be included. Furthermore, slight differences existed with regard to the reference tests used by the included articles. Finally, it needs to be mentioned that a cut-off, time-based division between early and late infections remains arbitrary and therefore subject to ongoing discussion [13].

CONCLUSION

The serum inflammatory markers CRP, LC and ESR are insufficiently accurate to diagnose late FRI. These markers cannot confirm or rule out the presence of FRI, and should therefore be used as a suggestive sign in the diagnosis of late FRI.

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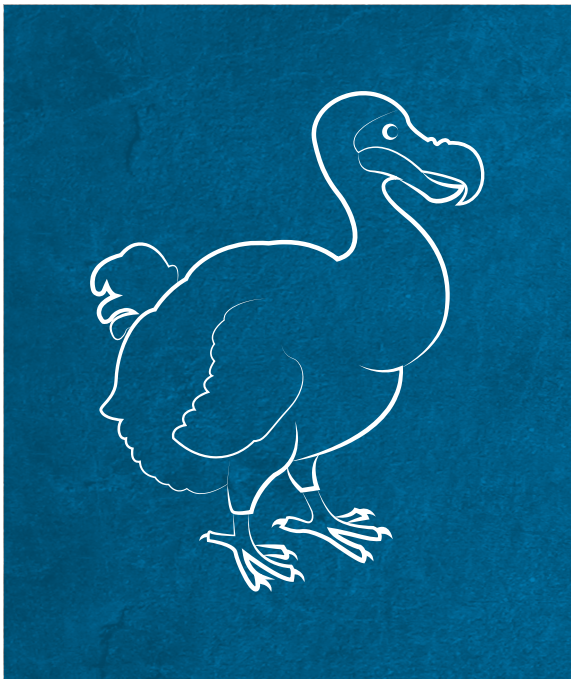
Appendix 1. Search strings for PubMed and Embase.

PubMed

(((surgical wound infection[MeSH] OR infectious bone disease[MeSH] OR infect*[tiab] OR osteitis[tiab]) OR infectious bone disease[tiab]) AND (bone fracture[MeSH] OR broken bone[tiab] OR fracture*[tiab] OR trauma*[tiab])) OR (osteomyelitis[MeSH] OR osteomyelitis[tiab])) AND (((biologic*[tiab] OR immunologic*[tiab] OR inflammat*[tiab] OR laboratory[tiab] OR serum[tiab]) AND (marker*[tiab] OR parameter*[tiab] OR mediator*[tiab]) OR (blood sedimentation[MeSH] OR c reactive protein[MeSH] OR leukocyte count[MeSH] OR inflammation mediators[MeSH] OR biomarkers[MeSH] OR blood sedimentation[tiab] OR sedimentation rate[tiab] OR c reactive protein[tiab] OR C-reactive protein[tiab] OR leukocyte*[tiab] OR leucocyte*[tiab] OR leukocytosis[tiab] OR leucocytosis[tiab] OR blood cell count[tiab] OR white blood cell*[tiab] OR CRP[tiab] OR ESR[tiab] OR immune marker*[tiab] OR erythrocyte sedimentation[tiab] OR biomarker*[tiab])) NOT (animals[MeSH] NOT humans [MeSH])

Embase

((('surgical infection'/exp OR infect*:ab,ti OR osteitis:ab,ti OR 'infectious bone disease':ab,ti) AND ('fracture'/exp OR 'broken bone':ab,ti OR fracture*:ab,ti OR trauma*:ab,ti)) OR ('chronic osteomyelitis'/exp OR osteomyelitis:ab,ti)) AND (((biologic*:ab,ti OR immunologic*:ab,ti OR inflammat*:ab,ti OR laboratory:ab,ti OR serum:ab,ti) AND (marker*:ab,ti OR parameter*:ab,ti OR mediator*:ab,ti)) OR ('erythrocyte sedimentation rate'/exp OR 'c reactive protein'/exp OR leukocyte/exp OR 'autacoid'/exp OR 'biological marker'/exp OR 'blood sedimentation':ab,ti OR 'sedimentation rate':ab,ti OR 'c reactive protein':ab,ti OR leukocyte*:ab,ti OR leucocyte*:ab,ti OR leukocytosis:ab,ti OR leucocytosis:ab,ti OR 'blood cell count':ab,ti OR 'white blood cell':ab,ti OR crp:ab,ti OR esr:ab,ti OR 'immune marker*':ab,ti OR 'erythrocyte sedimentation':ab,ti)) AND [humans]/lim



PART IV

MICROBIOLOGY

CHAPTER 9

Getting it right first time: the importance of a structured tissue sampling protocol for diagnosing fracture-related infections

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Submitted

ABSTRACT

Introduction. Fracture-related infection (FRI) is an important complication following surgical fracture management. Key to successful treatment is an accurate diagnosis. To this end, microbiological identification remains the gold standard. Although a structured approach towards sampling specimens for microbiology seems logical, there is no consensus on a culture protocol for FRI. The aim of this study is to evaluate the effect of a structured microbiology sampling protocol for fracture-related infections compared to ad-hoc culture sampling.

Methods. We conducted a pre-/post-implementation cohort study that compared the effects of implementation of a structured FRI sampling protocol. The protocol included strict criteria for sampling and interpretation of tissue cultures for microbiology. All intraoperative samples from suspected or confirmed FRI were compared for culture results. Adherence to the protocol was described for the post-implementation cohort.

Results. In total 101 patients were included, 49 pre-implementation and 52 post-implementation. From these patients 175 intraoperative culture sets were obtained, 96 and 79 pre- and post-implementation respectively. Cultures from the pre-implementation cohort showed significantly more antibiotic use during culture sampling ($P = 0.002$). The post-implementation cohort showed a tendency more positive culture sets (69% vs. 63%, $P = 0.353$), with a significant difference in open wounds (86% vs. 67%, $P = 0.034$). In all post-implementation culture sets causative pathogens were cultured more than once per set, in contrast to pre-implementation ($P < 0.001$). Despite stricter tissue sampling and culture interpretation criteria, the number of polymicrobial infections was similar in both cohorts, approximately 29% of all culture sets and 44% of all positive culture sets. Significantly more polymicrobial cultures were found in early infections in the post-implementation cohort ($P = 0.048$). This indicates a better yield in the new protocol.

Conclusion. A standardised protocol for intraoperative sampling for bacterial identification in FRI is superior than an ad-hoc approach. It has a positive effect on both surgeon and microbiologist by increasing awareness about the problem at hand. This resulted in more microbiologically confirmed infections and more certainty when identifying causative pathogens.

INTRODUCTION

Fracture-related infections (FRIs) are important complications following surgical fracture management. Not only do patients suffer from increased morbidity and prolonged hospital stay, they have to deal with increased healthcare costs and decreased quality of life [1-3].

Infection around fractures is suspected with the presence of clinical signs and symptoms and, if indicated, on additional laboratory tests and diagnostic imaging [4, 5]. Early FRIs tend to be easily recognisable by classic inflammatory symptoms such as redness, pain, warmth, and swelling [6]. Later infections may present more subtly, with delayed fracture healing and persisting tenderness as the only indication of a lingering infection [7]. This can make diagnosis more challenging, particularly as these infections are often caused by low-grade or difficult-to-culture organisms [8, 9].

Laboratory tests alone are insufficient for diagnosing FRIs [5, 6, 10]. Diagnostic imaging can aid in the diagnosis and provide information on status of fracture healing and extent of the infection, especially when chronic. However, studies on diagnostic accuracy of medical imaging for FRI are hampered by small patient series and insufficient data [11, 12]. The only incontestable gold standard to confirm the diagnosis of FRI is the presence of pathogens in deep surgical wounds [4, 5, 13].

Apart from establishing the presence or absence of FRI, an accurate microbiological diagnosis is crucial for an effective treatment. Treatment strategies rely heavily on microbiological findings, so it is of paramount importance that the right pathogens be identified [14]. First, targeting the right pathogen is important for effectiveness of the antibiotic treatment. Second, prescription of unnecessary broad-spectrum or incorrect antibiotics may lead to increased antimicrobial resistance [15].

Although the literature agrees that multiple specimens for microbiology should be taken to improve sensitivity and specificity, there is no agreement on the method to obtain these samples [9, 16]. Number of recommended cultures, but also source and technique used to obtain the specimen vary between studies and largely focus on prosthetic joint infection [6, 16, 17].

To introduce uniformity in sampling techniques and handling of samples taken intraoperatively from suspected infection sites, a standardised sampling protocol for FRIs was recently implemented in our hospital. This new approach was based on a tissue sampling protocol developed in the Bone Infection Unit, Oxford, UK for prosthetic joint

infections [16] and subsequently applied to osteomyelitis and fracture-related infections [9, 18]. The aim of this study is to evaluate the effect of a structured microbiology sampling protocol for fracture-related infections compared to ad-hoc culture sampling.

METHODS

Patient identification and ethical aspects

A cohort study was conducted in a single Level-1 trauma centre in the Netherlands, comparing a retrospective pre-implementation cohort with a prospective post-implementation cohort. Both cohorts included intraoperative cultures from patients with suspected or confirmed FRI, both early (<6weeks) and late (>6weeks) following the index operation. The first retrospective cohort included samples obtained between January and December 2014, based on a previous study [19] and served as a historical control group before implementation of the standardised sampling protocol. Possible subjects were retrospectively identified by screening surgical registries. Subjects from the second cohort were prospectively included from July to December 2017, starting at the implementation of the new standard-of-care sampling protocol for suspected or confirmed FRI, and thus became the research cohort. All patients with suspected infection following osteosynthesis were eligible for inclusion. Only intraoperative culture results from before and after implementation of the new sample protocol were included in the study to ascertain comparability of circumstances. The intraoperative cultures obtained during each revision operation were recorded for culture-based analyses, and are referred to in this paper as the 'culture set'. Patients from whom no intraoperative cultures were obtained were excluded from analysis. Baseline characteristics of both patients and cultures were collected from the electronic patient files (e.g. age, gender, affected bone, initial soft tissue damage, early/late infection, wound characteristics, etc.).

This study was reviewed and a waiver was provided by the local Medical Ethics Review Committee (METC), no: 18-147/C. All study procedures were performed in accordance with relevant guidelines and regulations. Patient-related data was processed and analysed anonymously.

Sampling approaches

Pre-implementation: There was no standardised sampling method for microbiological cultures. Ad-hoc intraoperative cultures were obtained. Number of cultures, method (e.g. tissue sample, swab, fluid, sonification), and use of antibiotics during sampling were decided by the treating surgeon.

Post-implementation: Samples for microbiological cultures were obtained following a standardised protocol. First, antibiotics during or prior to the sampling were avoided, preferably for at least two weeks. Preoperative antibiotic prophylaxes were withheld until all culture samples were obtained. A minimum of five deep-tissue or fluid samples were collected intraoperatively at the area of suspected FRI. Tissue samples were only obtained from infection-suspected tissue adjacent to the fracture, preferably from the implant-bone interface. Superficial or skin tissue or fluid samples were not considered for bacterial identification. Due to their low sensitivity, swabs were not allowed [20]. Sinus tracts were disregarded because of their low diagnostic value [21]. To minimise the risk of cross-contamination all samples were obtained with separate, sterile surgical instruments using a no-touch technique and transported in separate culture containers to the microbiology laboratory (Figure 1) [16]. Osteosynthetic implants were sent for sonication when appropriate. The request form was redesigned to emphasise that the patient had a suspected or confirmed FRI. Data on whether antibiotics had been administered prior to sampling and whether osteosynthetic implants remained in situ was mandatory clinical information that needed to be completed on the request form.

Figure 1. Example of a surgical instrument set to obtain non-contaminated tissue samples for microbiology and histology. This set can easily be assembled in any hospital and allows the surgeon to use clean, unused instruments for each specimen.

Microbiological procedures

Procedures within the microbiology laboratory did not change between the retrospective pre- implementation and prospective post-implementation periods.

Bone specimens were cultured in brain heart infusion broth with added hemin and nicotinamide adenine dinucleotide (X and V factors (BHXV)), if possible in thioglycolate enrichment broth (thio) incubated aerobically for 7 days, and if turbid subcultured in blood agar (BA) and chocolate agar (GC) (both 5% CO_2) or BA and brucella blood agar (BBA) (anaerobic incubation). Tissue specimens other than bone were homogenised using a bead-beater protocol.

Homogenised tissue specimens were cultured in BA (4 days, aerobically), GC (3 days 5% CO_2) McConkey agar (McC) (2 days, aerobically) and BBA (14 days, anaerobically), as well as in BHXV (7 days aerobically). Pus samples were cultured in BA (4 days, aerobically), GC (3 days 5% CO_2) McC (2 days, aerobically) and BBA (7 days, anaerobically).

Osteosynthetic materials were submerged in sterile sodium chloride 0.9% (w/v) by the operating surgeon in a sterile 'sonication jar' in the operating room. Upon arrival in the laboratory, sonication jars are sonicated for 1 min at maximum power (Bandelin BactoSonic). Uncentrifuged sonication fluid is cultured in BA and GC (4 days 5% CO_2), in BBA (7 days, anaerobically) and in thio (14 days aerobically).

Growth of different colonial morphologies was identified using MALDI-TOF MS (MBT Smart, research use only (RUO DB 6903) and security-related (SR) databases, Compass software, Bruker, Germany). Susceptibility testing was performed on isolates using Phoenix automated susceptibility testing (enterobacteriaceae, staphylococci, enterococci) or disk diffusion and/or E-test (all other isolates according to EUCAST methodology (disk diffusion) and manufacturer's instructions (Etest)). MIC values and disk diffusion growth inhibition zone diameters were interpreted according to EUCAST criteria [22].

Study outcomes

The primary outcome measure was the number of positive cultures. Incidences of the different pathogens were described. Incidences of polymicrobial and staphylococcal infections were compared. The definition of a causative pathogen was different in the pre- and post-implementation cohort.

Pre-implementation cohort: All reported isolates from tissue samples, swabs or fluids cultures were defined as causative pathogens. Culture of a single pathogen was regarded as significant and included in treatment considerations. This was in line with the clinical practice at that time.

Post-implementation cohort: Phenotypically indistinguishable microorganisms cultured from a minimum of two separately obtained samples from the same culture set were regarded as causative [5]. Again, swab samples were considered insufficiently reliable and were not allowed. Intraoperative fluid, tissue, and hardware cultures were considered as relevant cultures. Polymicrobial infection was defined as ≥2 pathogens cultured from at least two (out of five) specimens obtained in the same operation.

Differences between the causative pathogens and the incidence of polymicrobial infections between the two cohorts were analysed.

As a secondary outcome parameter, adherence to the protocol was described in the post-implementation cohort. Deviation from protocol was defined as sampling of less than five relevant cultures according to the method described above.

Statistical analysis

Patient characteristics and microbiological aspects of both cohorts were descriptive. Baseline characteristics were compared between the two patient cohorts. Differences in culture set results between the two protocols were analysed with the Pearson Chi-square test for categorical variables and the Mann-Whitney U-test for non-parametric continuous data. *P*-values <0.05 were considered significant. Analyses were conducted using SPSS 24 (IBM SPSS statistics, New York, NY).

RESULTS

Study population

In total 102 patients were eligible for inclusion in the study; 49 patients included in the retrospective 2014 pre-implementation cohort, which underwent the old sampling approach, and 52 patients included in the prospective 2017 post-implementation cohort, which underwent the new sampling protocol. Seventy-one patients were male, median age was 52 years (range 9-92). Tibial fractures were most often affected, at approximately 45%. In 43% of the patients it involved open fractures with varying degrees of soft-tissue injury. Plates were the hardware most often involved (51%) (Table 1).

Table 1. Baseline characteristics.

	Patient-based				Culture set-based			
	Protocol implementation				Protocol implementation			
	Pre (*n* = 49)	Post (*n* = 52)	*P*	Missing	Pre (*n* = 96)	Post (*n* = 79)	*P*	Missing
Gender, male	32	39	0.287[C]	0	65	61	0.163[A]	0
Age, years (range)	50 (18-92)	55 (9-83)	0.698[D]	0	50 (18-92)	57 (9-83)	0.201[D]	0
Body part			0.273[C]	0			**0.007[C]**	0
Humerus	3	4			3	4		
Radius/ulna	8	3			21	4		
Sternum	0	1			0	1		
Pelvic ring/acetabulum	4	5			5	12		
Femur	11	7			15	10		
Tibia/fibula	18	28			41	42		
Foot	5	4			11	6		
Soft-tissue injury								
Closed	28	30	0.961[A]	2	61	49	0.850[A]	2
Open, Gustilo classification			1.0[C]	4			0.693[C]	7
Gustilo I	3	4			4	5		
Gustilo II	4	5			6	5		
Gustilo IIIa	4	4			9	5		
Gustilo IIIb	4	6			6	12		
Gustilo IIIc	3	2			4	2		
Fixation type			0.509[C]	0			0.098[C]	0
Plate	25	27			45	44		
Screws	4	3			6	4		
Intramedullary nail	9	15			20	21		
K-wire/Tension Wire	3	2			5	2		
External fixator	3	2			14	3		
Prosthesis	1	0			1	0		
No fixation device	4	3			5	5		

Data are presented as number of cases or median (range), [A] Chi square,[B] Fisher's exact, [C] Linear-by-linear,[D] Independent samples median test Mann-Whitney U test.

The 102 patients underwent 175 separate operations in which samples for microbiology were obtained (sampling moments); 96 from the pre-implementation and 79 from the post-implementation cohort. Comparison based on culture set characteristics showed significantly more culture sets obtained from the radius and ulna ($P = 0.014$) pre-implementation and comparable soft-tissue injuries, but slightly more grade IIIb open fractures post-implementation($P = 0.693$). In both cases this could be attributed to prolonged infection in selected cases (two and three patients respectively), with multiple revision surgeries and thus multiple culture sets.

Culture approach characteristics

Culture characteristics per cohort are shown in Table 2. One case from the post-implementation cohort was excluded from further analysis due to gross protocol violation, as only one intraoperative tissue sample was obtained. Early and late infections were equally represented in both cohorts. In both cohorts early and late infections were equally represented (55 % pre-implementation vs. 56% post-implementation ($P = 0.799$)). In the post-implementation cohort significantly more samples were obtained from closed wounds (46% vs. 53%, $P = 0.030$). The tendency towards more pre-implementation cultures from vacuum-dressed wounds was largely explained by the patients with prolonged infection discussed above. Secondly, in the post-implementation cohort surgeons were strongly discouraged from culturing wounds covered by vacuum assisted closure (VAC) dressings, as this merely reflects the superficial bacterial colonisation of the wound and the VAC sponge. As per protocol, there was a difference in the incidence of antibiotics being administered at the moment of culturing, with a significant lower incidence post-implementation ($P = 0.002$). The median number of relevant samples obtained pre- and post-implementation differed significantly: 1 (range 0-5) pre-implementation and 5 (range 2-9) post-implementation ($P<0.001$). As stated, pre-implementation 'non-relevant' intraoperative cultures from swabs and sinus tracts were included for further analysis, as this was clinical practice at that time. Median number of all cultures combined obtained in the pre-implementation cohort was 1 (range 1-7).

Table 2. Infection and culture characteristics of culture sets.

	Protocol implementation			
	Pre (n = 96)	**Post (n =78)**	***P***	**Missing**
Infection type			0.799[A]	1
Early (<6w after index surgery)	53	44		
Late (>6w after index surgery)	43	33		
Soft-tissue status at time of surgery for suspected infection			**0.030[C]**	1
Closed	44	41		
Dehiscent	21	23		
Draining fistula	10	10		
VAC in situ	20	4		
Antibiotics given during sampling	53	25	**0.002[A]**	
Median number of samples	1 (1-7)	n/a	**n/a**	0
Median number of relevant samples	1 (0-5)	5 (2-9)	**<0.001[D]**	0

n/a not applicable; VAC = vacuum dressing.
Data are presented as number of cases. [A] Chi square, [B] Fisher's exact, [C] Linear-by-linear,[D] Independent samples median test Mann-Whitney U test

Twenty post-implementation culture sets deviated from the protocol, all based on the number of relevant cultures obtained during each set. Ten sample sets contained four samples, five sets with three samples, and five sets with only two samples. Except for the number of cultures obtained, culture set characteristics did not differ between the sets obtained according to the protocol and the sets that deviated from it.

Microbiology

Bacteria most often found were *Staphylococcus aureus* (23%), *Coagulase-negative Staphylococci* (19%), *Enterococcus* species (8%), *Corynebacterium* species (7%), *Enterobacter* species (7%), and *Pseudomonas aeruginosa* (7%). The same bacteria were also most often cultured in cases of polymicrobial cultures. Of the 200 bacteria cultured, 134 (67%) were found in a polymicrobial culture. The difference in frequency of aerobic and anaerobic bacteria found was largely due to the high number of *S. aureus*. An overview of the identified bacteria is shown in Table 3.

Table 3. Microbial identification in early and late culture sets.

Microorganism (MO)	Total *n = 200*	PolyMO *n = 134*	Early *n = 144*	Late *n = 56*
Staphylococcus aureus	46	18	32	14
CoNS	37	24	30	7
Enterococcus species	15	14	12	3
Corynebacterium species	14	13	12	2
Enterobacter species	13	9	11	2
Pseudomonas aeruginosa	13	9	5	8
Escherichia coli	10	6	5	5
Streptococcus	9	9	7	2
Klebsiella species	5	4	2	3
Finegoldia magna	5	5	4	1
Acinetobacter species	4	3	1	3
Clostridium species	3	2	2	1
Proteus mirabilis	3	3	3	0
Bacteroides fragilis	3	3	3	0
Peptoniphilus harei	3	3	2	1
Propionibacterium species	3	1	2	1
Fusobacterium nucleatum	2	1	1	1
Serratia marcescens	2	0	1	1
Actinobaculum schaalii	1	1	1	0
Cedecea davisae	1	1	1	0
Citrobacter koseri	1	1	1	0
Granulicatella adiacens	1	1	1	0
Kytococcus schroeteri	1	0	1	0
Parvimonas micra	1	0	0	1
Porphyromonas spp	1	1	1	0
Prevotella denticola	1	1	1	0
Rhodococcus equi	1	0	1	0
Lactobacillus	1	1	1	0

The total number is the combined values of early and late infections. Infections are regarded as early when manifested within 6 weeks after initial fracture fixation surgery, late infections manifested after 6 weeks. PolyMO is the number of times the bacterium is cultured as part of a polymicrobial culture.

In addition to culture set characteristics, no differences were found in microbiological aspects between protocol deviations and non-deviations in the post-implementation cohort. Hence all sets were analysed together, as this represents clinical practice. Microbiology results from culture sets are summarised in Table 4. Inherent to the sampling approach, there were significantly more positive cultures per culture set post-implementation (4 vs. 1, P <0.001). In total, the overall culture set results came back negative in 60 cases, 36 pre-implementation and 24 post-implementation (P = 0.353). From the positive cultures in the pre-implementation cohort, more than one culture per set identified the same pathogen in 42% of the culture sets, compared to 100% in the post-implementation cohort (P < 0.001). Implementation of a sampling protocol for FRI did not lead to decreased numbers of polymicrobial (33% post-implementation vs. 25% pre-implementation, P=0.381) or *Staphylococcus aureus*-positive cultures (27% post-implementation vs. 26% pre-implementation, P=0.763) in the total groups. Sub-analysis of open (dehiscent wounds, fistulas and wounds temporarily covered with vacuum dressings) and closed wounds at the time of culture sampling showed an additional difference in negative culture results, with significantly more negative results found pre-implementation in patients with open wounds (P=0.034). No additional differences were found in closed wounds. Sub-analysis of early and late infection showed significantly more polymicrobial infections post-implementation in early infections (P=0.048) (Table 4).

Because higher numbers of positive cultures were obtained per culture set post-implementation, we calculated the ratio of positive cultures per absolute number of samples obtained. Although not significant, this was also higher in the new cohort than in the old cohort, 0.68 vs. 0.59 (P=0.175) positive culture per sample obtained. Hence, a higher number of samples obtained after implementation of the sampling protocol did not seem to be the only reason for higher overall positive culture results.

Table 4. Microbiological characteristics of all culture sets.

	Total			Cultures from open wounds			Cultures from early infections		
	Protocol implementation			Protocol implementation			Culture Protocol implementation		
Microbiological variable	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***
All cultures	***n* = 96**	***n* = 78**		***n* = 51**	***n* = 37**		***n* = 53**	***n* = 44**	
Median number of positive cultures	1 (0-6)	3.5 (0-8)	<0.001[D]	1 (0-6)	4 (0-7)	**<0.001[D]**	1 (0-6)	4 (0-7)	**<0.001[D]**
Negative culture set	36	24	0.353[A]	17	5	**0.034[A]**	18	6	**0.021[A]**
Positive culture set	60	54		34	32		35	38	
Positive cultures	**n = 60**	**n = 54**		**n = 34**	**n = 32**		**n = 35**	**n = 38**	
Same pathogen >1 cultures per set	25	54	**<0.001[A]**	12	32	**<0.001[A]**	15	38	**<0.001[A]**
Median number of pathogens	1 (1-5)	1 (1-6)	0.0446[D]	1 (1-5)	2 (1-6)	0.504[D]	1 (1-5)	2 (1-6)	0.170[D]
Median number of species	1 (1-5)	1 (1-6)	0.900[D]	1 (1-5)	1.5 (1-6)	0.686[D]	1 (1-5)	2 (1-6)	0.532[D]
Staphylococcus aureus (mono/polymicrobial)	25	21	0.763[A]	14	15	0.641[A]	17	15	0.434[A]
Polymicrobial infections	24	26	0.381[A]	14	17	0.331[A]	14	24	**0.048[A]**
Monomicrobial infections	36	28		20	15		21	14	
Monomicrobial cultures	**n = 36**	**n = 28**		**n = 20**	**n = 15**		**n = 21**	**n = 14**	
Staphylococcus aureus	16	12	0.899[A]	10	8	0.845[A]	9	7	0.678[A]
Other monomicrobial infections	20	16		10	7		12	7	

Data are presented as number of cases or median (range). [A] Chi square,[B] Fisher's exact, [C] Linear-by-linear, [D] Independent samples median test Mann-Whitney U test.

DISCUSSION

This study aimed to evaluate the effect of a structured sampling protocol for FRI. The new structured sampling protocol performed better at identifying pathogens with more positive cultures. As in all positive culture sets the causative pathogen had to be cultured at least in two different cultures, increased microbiological certainty was achieved. Despite these stricter criteria for causative pathogens no decrease in number of polymicrobial cultures or number of *Staphylococcus aureus*-positive cultures was found post-implementation.

Our hypothesis was that the *ad hoc* (pre-implementation) sampling method is inferior to a structured and standardised protocol (the post-implementation method) and this hypothesis is positively confirmed by the results of this study.

We found no difference in the species of microorganisms cultured between the two cohorts. We expected to find less polymicrobial infection as a result of the stricter criteria for causative pathogens (growth in at least two separate cultures and prevention of cross-contamination with skin flora). This was not the case, with even more polymicrobial infections found in the post-implementation cohort in the early infections. A possible explanation is that the higher number of relevant samples leads to increased opportunities to identify pathogens. Meanwhile, more precise sampling and stricter criteria will lower the possibility of identification, thereby partially equalling out the measurable effect of the protocol in this cohort. Overall, we found high numbers of polymicrobial infections (25% and 36% of all cultures pre- and post-implementation respectively). This is on the upper limit of what is described in the literature. The incidence of polymicrobial infection following osteosynthesis is described to be around 15-27% [6, 23]. This may be explained by the setting of the study. Ours being a Level-1 trauma centre, most of the trauma patients admitted to our hospital are severely injured. This may contribute to the high number of fractures accompanied by soft-tissue injury (Table 1). Although not proven, one can argue that severe soft-tissue injury leads to more complex pathogen patterns and multiple operations, which can subsequently result in more complex infections. Additionally, severe injury affects the host's immune system, making it susceptible to bacterial infections [24]. Accurate diagnosis of polymicrobial infections is necessary for proper antibiotic treatment, as such infections are associated with worse outcome in orthopaedic patients [25]. However, argued above, a lack of structured sampling with strict microbiological definitions can skew data on polymicrobial infections in other studies. Thus, making it hard to directly compare results.

In our study, we observed a significant difference between the number of positive cultures per operation. We also observed a trend towards more positive culture sets under the structured protocol, with significantly more positive culture sets in open wounds. We feel that the difference in positive cultures is a result of the protocol as a whole. Multiple relevant tissue sampling, avoidance of antibiotics [26] and improved information transfer on the request form contributed to this result. Atkins et al [16] recommend a minimum of five protocolised tissue samples for prosthetic joint infections (PJI). They compared several tissue cultures to histological specimens of PJI and found 65% positive cultures in histologically confirmed infections. Based on this data they calculated that five or six cultures should be obtained to reliably diagnose infection. By contrast, Peel et al. [27] and Bémer et al. [23] showed that fewer specimens seeded on more culture media was as effective as five specimens on fewer media. However, as they themselves argue, this method is mostly recommended for subacute or chronic infections and is hard to extrapolate to acute infections because of pathogenicity and growth characteristics of bacteria involved. The overwhelming clinical presentation in acute infections is due to more bacteria, which should simplify bacterial identification and decrease the need for repetitive tissue sampling [23, 27, 28]. Nevertheless, it takes a couple of days for the bacterial burden to reach maximum levels [29]. So when suspicion of infection is raised early on, bacterial burden may still be relatively low and therefore the proposed method still applies. In order to establish a clear and non-confusing protocol with minimal opportunities for errors we chose a pragmatic recommendation of a minimum of five samples in every type of FRI.

Earlier studies show lower sensitivity and specificity of swab cultures compared to extensive cultures of tissue samples or scrapings from biomaterial surfaces [20, 29-31]. And yet, the same studies also show increased numbers of positive cultures with prolonged incubation time for bacterial growth [30, 32]. Although the generally recommended incubation time of 7 days is also maintained in our protocol for broth cultures, a prolonged incubation of 14 days is recommended in specific cases [32]. This might be of interest when slow-growing microorganisms are to be expected. The introduction of a customised microbiological request form raised awareness among all parties involved. This resulted in more careful sampling by the surgeon and more thorough culture procedures with different media and prolonged incubation time by the microbiologist. Implant-related infections are known to be associated with anaerobic bacteria (especially in open fractures) [33] as well as frequent infection by *Staphylococcus aureus* [34]. Both pathogens require special attention in interpreting culture data. Anaerobic and aerobic pathogens require different culture conditions and *S. aureus* can enter a stationary growth phase in biofilms, resulting in impaired bacterial growth. By implementing a structured request form in the new protocol, sample

origin (e.g. deep tissue, bone) and the problem at hand (infection near orthopaedic implants) are emphasized. Thereby, triggering the microbiologists to prolong culture time if necessary and minimize 'missed' pathogens. Optimising culture conditions by growing bacteria in blood culture flasks has been proposed to increase sensitivity of culture samples and may improve results even further [29]. We did not yet implement this recommendation in our hospital, but to correct for this, all removed implants were sent for sonication to break down the biofilm prior to culturing.

There are some drawbacks to our study. First we compared two different approaches, with differences in their definition of FRI and causative pathogen. For purposes of this study, to evaluate the effect of a sampling protocol compared to previous clinical practice, this did not form a problem. Unfortunately, this does result in the inability to analyse the separate elements incorporated into the new standardised protocol. Second, in the pre-implementation cohort swabs and tissue samples were obtained from every redebridement surgery, even VAC-system replacements. Because of stricter sampling criteria in the post-implementation cohort only uncontaminated, deep samples were obtained. This could have lead to more false culturing outcome in the pre-implementation cohort. Again, because this was customary at that time and (antibiotic) treatment was based on the microbiology results of each sample obtained in the pre-implementation cohort, we used these results specifically to compare old versus new standard of care. As discussed above, chronic and acute infections may differ in the difficulty of bacterial identification. This may skew results. However, the percentage of patients with early versus chronic infections did not differ in the cohorts compared in this study. Last, it is possible that with a prolonged inclusion time and thus more included patients, more (significant) differences in outcome could be found. Remarkably however, is that relatively more infections are included in the post-implementation cohort (same number, short inclusion period). We feel this is due to improved pre-hospital triage, leading to increased numbers of patients with complex extremity injuries referred to our hospital over the years [35].

In conclusion, even with stricter criteria for pathogen identification a structured tissue sampling approach for fracture-related infection leads to increased microbiological identification with more certainty of causative pathogens. This ensues from both the improved and standardised sampling technique and the customised culture request form. It has a positive effect on both surgeon and microbiologist by increasing awareness about the problem at hand. This results in a more complete and honest overview of the infected tissue, more trustworthy culture results, and consequently a more targeted treatment. Future research should focus on cost effectiveness of such a protocol and possible alternatives in microbial culture or pathogen identification techniques.

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CHAPTER 10

Accuracy of tissue and sonication fluid sampling for the diagnosis of fracture-related infection: a systematic review and critical appraisal

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ABSTRACT

Introduction. Intraoperatively obtained peri-implant tissue cultures remain the standard for diagnosis of fracture-related infection (FRI), although culture-negative cases may complicate treatment decisions. This paper reviews the evidence on sonication fluid and tissue sampling for the diagnosis of FRI.

Methods. A comprehensive search in Pubmed, Embase and Web-of-Science was carried out on April 5, 2018, to identify diagnostic validation studies regarding sonication fluid and tissue sampling for FRI.

Results. Out of 2624 studies, nine fulfilled the predefined inclusion criteria. Five studies focused on sonication fluid culture, two on polymerase chain reaction (PCR) and two on histopathology. One additional histopathology study was found after screening of reference lists. There is limited evidence that sonication fluid culture may be a useful adjunct to conventional tissue culture, but no strong evidence that it is superior or can replace tissue culture. Regarding molecular techniques and histopathology the evidence is even less clear. Overall, studies had variable 'gold standard' criteria for comparison and poorly reported culture methods.

Conclusions. Scientific evidence on sonication fluid and tissue sampling, including culture, molecular techniques and histopathology for the diagnosis of FRI is scarce. It is imperative that lab protocols become standardized and uniform diagnostic criteria, as recently published in a consensus definition, implemented.

INTRODUCTION

Approximately 14% of all trauma admissions suffer from at least one complication [1]. The incidence of infectious complications that involve a fracture, i.e. fracture-related infection (FRI), can range from 1 to 2% after internal fixation of closed fractures, up to 30% after open fractures [2, 3]. FRI can have serious consequences, with impairment of patient function and even amputation of the affected limb [4]. From a socio-economic point of view the associated costs cannot be neglected, as treatment often involves a prolonged hospital stay, long-term intravenous antibiotic therapy and additional revision surgeries [5]. In open tibial fractures, infection doubles the length of hospital stay and increases direct costs of care by 60% [6]. Regarding treatment of FRI, one of the most difficult hurdles to tackle is the presence of a biofilm [2]. Bacteria attach to the surface of the orthopaedic implant or fracture fixation device and produce extracellular matrix, making them inaccessible to the host immune system as well as to most antibiotic drugs [2]. To increase the probability of successful treatment, an early and accurate diagnosis is of utmost importance [7].

Although these issues are well known, studies solely focusing on FRI are scarce, and therefore current diagnostic and treatment concepts for FRI are primarily based on those for prosthetic joint infection (PJI) [2]. Although there are similarities, important distinctions should be made between FRI and PJI, with the presence of a fracture and soft tissue damage being the most important [2]. Multiple definitions are available for PJI [8, 9]. For FRI, a consensus definition was recently proposed, utilizing two levels of certainty around diagnostic features: confirmative and suggestive [10]. Two of the four confirmatory (diagnostic) criteria are: phenotypically indistinguishable pathogens identified by culture from at least two separate intraoperative tissue or implant (sonication) specimens and the presence of microorganisms in intraoperative tissue taken during an operative intervention, as confirmed by histopathological examination. Deep tissue cultures, obtained from intraoperative samples remain the gold standard for diagnosis. Data on other techniques such as culture of sonication fluid from hardware, PCR and histopathology (i.e. presence of polymorphonuclear neutrophils (PMN's) are less clear with respect to the diagnosis of FRI [2, 10].

This systematic review provides an overview of validation studies regarding sonication fluid cultures, molecular techniques and histopathology as diagnostic criteria for FRI. The main hypothesis is that data focusing on these techniques in FRI is limited and well-designed, prospective clinical studies are necessary to improve our knowledge regarding this topic.

METHODS

On April 5 2018, with the help of a biomedical reference librarian (TV), a comprehensive literature search was performed in Embase, Pubmed and Web-of-Science. The methodology of this study was written following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Only articles in English, French or German language were included. The search strings are attached as supplementary material. Two reviewers (JO and WJM) screened all articles and in case of indecision a third reviewer was consulted (MD). Articles were first screened on title and abstract after which the full-text of the included articles were reviewed. A detailed description of eligibility criteria is listed in Table 1. Inclusion criteria were original research papers validating diagnostic lab methods for FRI of long bones. Studies looking into both PJI and FRI were included. Reasons for exclusion were animal studies, unavailable full-texts, reviews, studies evaluating cultures obtained during acute open fracture management, case reports, poster presentations, conference abstracts and articles published before 1990.

RESULTS

A total of 10 validation studies corresponded to the eligibility criteria (Fig. 1). Of these studies, five focused on sonication fluid culture [11-15], two on PCR of either tissue swabs or sonication fluid [16, 17], and three on histopathology [18-20]. One of the sonication studies [15] investigated various diagnostic modalities, including histopathology. One of the PCR studies included sonication fluid culture as well as histopathology [17]. No studies validating other diagnostic tests for sonication fluid or tissue samples (i.e. FISH) met the eligibility criteria displayed in Table 1.

Table 1. Detailed eligibility criteria for the systematic review.

	Inclusion criteria	**Exclusion criteria**
Target population	Patients who suffered musculoskeletal trauma of the long bones and consequently developed FRI.	Studies limited to patients with PJI Animal studies Spine studies
Tests	Sonication fluid tests • Sonication fluid culture • Sonication fluid PCR • Sonication fluid Gram's stain Peri-implant tissue tests • Tissue cultures • Molecular methods - PCR - Fluorescent in situ hybridization (FISH) • Histopathology	Serum marker tests Imaging modalities
Types of study	Validation studies: original research papers assessing • Sensitivity and specificity of tests • Positive predictive value (PPV) and negative predictive value (NPV) of tests	Case reports, reviews, language other than English, French or German, no full-text, poster presentations, conference papers, commentaries, expert opinions, articles older than 1990, studies evaluating cultures obtained during open fracture management studies

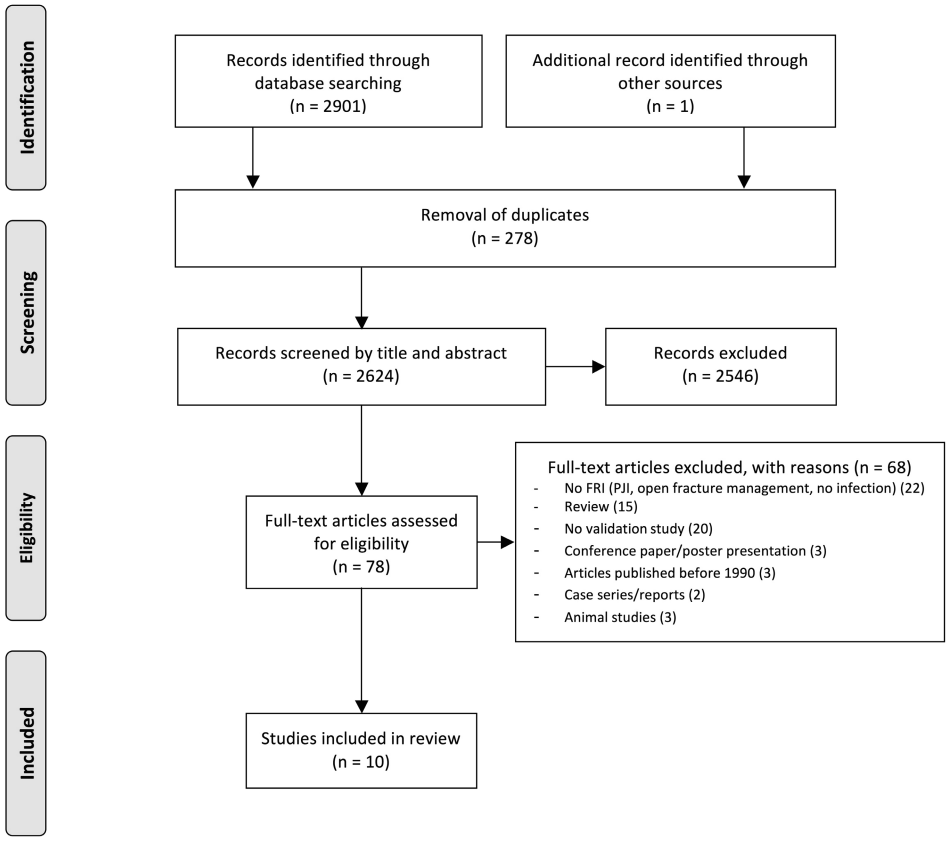

Figure 1. Flowchart of the systematic search and selection process following the PRISMA statement. FRI: Fracture-Related Infection.

Sonication

The studies evaluating the diagnostic value of sonication fluid cultures are summarized in Table 2. Most of these studies included both patients with prosthetic joints and patients with fracture fixation devices and, overall, referred to a PJI definition for infection [11, 13-15]. One study by Yano et al. focused on patients with fracture fixation devices, including spinal implants (13.8%), and referred to a customized definition for osteosynthesis-associated infection [12]. All studies used diagnostic criteria from the definition they provided as a reference to diagnose infection and all included tissue cultures as a comparator. Regarding incubation time and number of tissue samples taken, lab and sampling protocols differed between studies.

One study by Puig-Verdié et al. [11] could not detect a significant difference between sonication fluid culture and tissue culture for FRI patients. Six percent of patients in this study received antibiotics before surgery (antibiotics were stopped within 1 to 22 days prior to surgery). Fracture fixation devices were assessed separately from spinal implants in this study [11]. Yano et al. focused on osteosynthesis-associated infection and found a significantly higher sensitivity for sonication fluid culture compared to tissue culture. This difference could not be found for specificity. Overall, 31.2% of patients in this study received antibiotics within 14 days prior to the surgery [12]. A higher sensitivity rate was also found for sonication fluid cultures versus tissue cultures in the study by Portillo et al. However, this study did not assess test accuracy for fracture fixation devices separately from prostheses and did not specify which types of fixation material were included. Fifty-six percent of study subjects received antibiotics in the 14 days prior to surgery [13]. Three studies reported a sub-analysis on patients who received preoperative antibiotic treatment. Except for the study performed by Yano et al., all sub-analyses left the type of implant (i.e. prosthesis or fracture fixation device) out of consideration. For the subgroup of patients who received antibiotics at the time of sampling, all three studies concluded a higher sensitivity rate for sonication fluid cultures compared to intraoperative tissue cultures [11-13]. The studies by Esteban et al. [14] and Holinka et al. [15] did not assess fracture fixation devices separately from prostheses. Both studies had small sample sizes, Esteban et al. included 13 patients and Holinka et al. included 6 patients with fracture fixation devices. Esteban et al. did not report significance levels or number of patients receiving preoperative antibiotic therapy. This study found a low specificity rate (50%) for sonication fluid cultures, which was attributed to possible contamination and or use of multiple culture media [14]. Holinka et al. did not provide overall sensitivity and specificity rates but reported them separately for patients who did or did not receive preoperative antibiotics. The authors compared sonication fluid cultures with Gram's stain of centrifuged sonication fluid, tissue cultures and histopathology. They concluded that the sensitivity of sonication fluid cultures was significantly higher than that of tissue cultures with two or more positive cultures yielding the same microorganism. In patients who received antibiotics preoperatively, no statistically significant difference was found between tissue cultures with two or more positive cultures yielding the same organism and sonication fluid cultures [15]. The study by Renz et al. [17], detailed in the molecular techniques section (Table 3), found no significant difference between the sensitivity of sonication fluid cultures (84%) and the sensitivity of tissue cultures (66%).

Table 2. Studies on sonication fluid culture of orthopaedic implants.

Author	N_T	N_{Fix}	Test	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Reference criteria	Incubation time	number of tissue samples
Puig-Verdié *et al.* 2013	317	101	SFC	93,3	97,2	93,3	97,2	Definition: PJI Diagnostic criteria: ≥1 of the following: - Purulence synovial fluid or surrounding implant - Sinus tract communicating with implant	Aerobe: 7 days Anaerobe: 7 days	
			TC	76,7	100	100	91	- Histopathology: acute inflammation (≥5 PMN's/HPF) - Positive culture (SFC or TC) with clinical signs	Aerobe: 7 days Anaerobe: 7 days	5
Yano *et al.* 2014	180	180	SFC	90,4	90,9	95,8	80,6	Definition: customized definition for FRI Diagnostic criteria: ≥1 of the following: - Open wound exposing fracture and/or osteosynthesis with purulence	Aerobe: 7 days Anaerobe: 14 days	
			TC	56,8	96,4	97,3	49,7	- Intraoperative tissue with visible purulence - Draining fistula communicating with implant - Histopathology: acute inflammation in intraoperative osteosynthesis tissue	Aerobe: 7 days Anaerobe: 14 days	>1
Portillo *et al.* 2014	75	30	SF-HC	100	100	100	100	Definition: PJI Diagnostic criteria: For FRI cases: ≥1 of the following: - Purulence of site aspirate or at surgical site	Aerobe: 7 days Anaerobe: 14 days	
			SFC	87	100	100	88	- Tract communicating with implant - Clinical infection signs In addition, for PJI: - Histopathology: acute inflammation - Acute inflammation in preoperative joint aspiration	Aerobe: 7 days Anaerobe: 14 days	
			TC	59	100	100	69		Aerobe: 7 days Anaerobe: 14 days	not mentioned

Table 2. (continued)

Esteban *et al.* 2008	31	13	SFC	94,7	50	75	85,7	Definition: PJI Diagnostic criteria: ≥1 of the following: - Fistula - Purulent wound discharge - Intraoperative periprosthetic purulence - Sinus tract communicating with prosthesis - Laboratory findings (CRP and ESR) - Radiological signs - Gamma scan findings	Aerobe: 7 days Anaerobe: 7 days	
			TC	84,2	100	100	81,8		no protocol provided	3 to 5
Holinka *et al.* 2010	60	6	SFC					Definition: PJI Diagnostic criteria: ≥1 of the following: - ≥2 cultures of joint aspirates or tissue cultures yielding the same microorganism - Purulence surrounding the prosthesis - Histopathology: acute inflammation - Sinus tract communicating with prosthesis	Aerobe: 5 days Anaerobe: 7 days	
			AB-	83,3	95	93,8	86,4			
			AB+	65,9	94,7	96,4	56,3			
			TC1						Aerobe: 5 days Anaerobe: 7 days	at least 3
			AB-	72,2	95	92,9	79,2			
			AB+	57,5	95	95,8	52,8			
			TC2							
			AB-	61.1	95	91,7	73,1			
			AB+	42,5	95	94,4	45,2			
			SF-GS							
			AB-	76,5	100	100	83,3			
			AB+	55	100	100	52,6			
			HP	95	100	100	99,9			

Sensitivity and specificity are reported as they were calculated for all cases, without differentiating between cases who received preoperative antibiotics and those who did not receive antibiotics preoperatively, except for the study by Holinka *et al,* which did not provide overall values. N_t: total number of study participants, N_{Fix}: total number of participants with fracture fixation devices, Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value, PMN's: Polymorphonuclear neutrophils, HPF: high power field, SFC: sonication fluid culture, TC: tissue culture, TC1: single tissue culture, TC2: ≥2 tissue cultures yielding the same microorganism, HC: hemoculture, SF-GS: Gram's stain from the centrifuged sediment of the sonication fluid, HP: histopathology, AB-: without preoperative antibiotics, AB+: with preoperative antibiotics, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate.

Table 3. Data on the diagnostic performance of PCR.

Author	N_T	N_{fix}	Test	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Reference
Omar *et al.* 2016	62	62	PCR	69	64	90	30	Definition: customized definition for FRI Diagnostic criteria: ≥1 of the following: • Sinus tract or open wound communicating with the implant • Purulence encountered intraoperatively • Two out of three tissue cultures positive with the same pathogen
			TC	92	82	96	69	
Renz *et al.* 2018	51	51	SF-PCR	71	92	96	52	Definition: consensus definition for FRI Diagnostic criteria: ≥1 of the following: • Macroscopic purulence around the implant and/or presence of a sinus tract communicating with the implant and/or implant on view • Presence of inflammation in peri-implant tissue, as defined by the pathologist • Positive culture of peri-implant tissue or sonication fluid
			SFC	84	100	100	68	
			TC	66	100	100	40	
			HP	74	100	100	33	

N_T: total number of study participants, N_{Fix}: total number of participants with fracture fixation devices, Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value, TC: tissue cultures, SF-PCR: sonication fluid PCR, SFC: sonication fluid culture, HP: histopathology.

Molecular techniques

Two studies were identified regarding molecular techniques, which are summarized in Table 3. Omar et al. [16] investigated diagnostic performance of 16S rRNA PCR on swabs taken from the implant surface versus standard tissue cultures. This study provided a customized definition for FRI that was used as a reference. Only subjects undergoing revision surgery of fracture fixation devices were included. Tissue cultures were found superior to swab PCR, as a significantly higher area under the ROC curve was associated with tissue cultures. This study obtained 3 to 5 tissue samples for tissue cultures, but did not provide details on incubation time. Renz et al. [17] assessed the diagnostic performance of multiplex PCR on sonication fluid. This study applied the diagnostic criteria from the recently published consensus definition for FRI [10]. No statistically significant differences were found between tissue cultures and sonication fluid cultures or between tissue cultures and sonication fluid PCR. Specificities exceeded 90% for all tests. For tissue cultures, a minimum of 3 tissue samples was obtained. Tissue cultures

were incubated for 7 days (aerobic cultures) or 14 days (anaerobic cultures). Sonication fluid cultures were incubated for 14 days (aerobic cultures as well as anaerobic cultures). Spinal implants were included in this study as well [17].

Histopathology

Three studies assessed diagnostic accuracy of histopathology [18-20]. Results are displayed in Table 4. Simpson et al. used a customized definition for infected non-union based on clinical and microbiological results [18]. Chadayammuri et al. referred to the Centers of Disease Control definition for osteomyelitis [19]. Egol et al. did not refer to a definition for osteomyelitis [20]. In the study by Simpson et al., tissue cultures were incubated aerobically and anaerobically for 7 days. Histopathology criteria were provided. The authors reported a diagnostic accuracy of 91% for histopathology of fracture non-union. In cases where the diagnosis could not be determined based on microbiological and clinical criteria, histopathology proved to be a useful adjunct [18]. The second study, by Chadayammuri et al., compared diagnostic accuracy of soft tissue histopathology with that of deep wound swab cultures, relative to open bone biopsy and culture. Soft tissue histopathology was performed in 61 out of 159 cases with post-traumatic osteomyelitis. Swab cultures were incubated aerobically and anaerobically for 5 days. No criteria or threshold values were provided for histological assessment. The authors concluded poor results regarding sensitivity and specificity for swab cultures as well as for soft tissue histopathology, relative to open bone biopsy and culture [19]. Finally, the study by Egol et al. was identified by going through reference lists. This study compared frozen section histopathology to permanent section histopathology, with 'positive intraoperative tissue culture' as the reference test. Based on this reference test, six out of 51 cases were diagnosed as infected. Histopathology criteria were provided. No information on tissue culture methods was provided, neither was the lab protocol to obtain frozen or permanent sections. Frozen section analysis showed poor ability to predict the presence of indolent infection. Results from permanent section histopathology were slightly better [20]. The study by Holinka et al., which is detailed in the sonication section (Table 2), looked into the diagnostic accuracy of sonication fluid cultures but included histopathology as well. The authors reported a sensitivity of 95% and a specificity of 100% for histopathology. The utilized criterion for histopathology was the presence of acute inflammation, without further specification [15]. Renz et al. included histopathology as well [17]. This study is described in more detail in the molecular techniques section of this paper (Table 3). A sensitivity and specificity of 74% and 100% was found, respectively. The authors referred to the histopathology criteria proposed by Ochsner et al., with the presence of bone necrosis, damaged soft tissue surrounding it and the penetration of microorganisms as the most prominent features for osteomyelitis [21].

Table 4. Data on diagnostic performance of histopathology.

Author	N_T	Histopathology Criteria	Test	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Reference
Simpson *et al.* 2002	60 ununited fracture cases	Highly suggestive: >1 PMN/HPF on average after examination of 10 HPF Definite infection: Organisms seen on Gram's staining	Soft tissue histopathology	87	100	100	78	Definition: positive microbiological and clinical diagnosis. Microbiological diagnosis based on the culture of ≥5 deep specimens of tissue from the non-union site. Positive result if at least two separate specimens yielded the same organism. Clinical diagnosis: ≥1 of the following: • Open wound or draining sinus pre-operatively • Purulence encountered intraoperatively
Chadayammuri *et al.* 2017	159 cases with post traumatic osteomyelitis of the long bones	Not provided	Soft tissue histopathology	69,8	38,9	/	/	Open bone biopsy and culture
			Deep wound swab culture	66	28,1	/	/	
Egol *et al.* 2002	51 cases with delayed or non-union	Infected: >10 PMN's/HPF Not infected: <5 PMN's/HPF Equivocal: 5-10 PMN's/HPF	Frozen section histopathology	0	98	0	88	Positive intraoperative tissue culture
			Permanent section histopathology	33	95	50	91	

N_T: total number of study participants, Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value, PMN('s): polymorphonuclear neutrophil(s), HPF: high power field, CDC: Centers for Disease Control, HC: hemoculture.

DISCUSSION

FRI is a serious complication following musculoskeletal trauma surgery. Diagnosis of FRI is challenging and is generally based on the combination of clinical, laboratory, histopathology, imaging and pathogen isolation approaches [3,10,12]. Tissue cultures are widely used as a standard test, however, evidence for the use of tissue cultures and other diagnostic tests in the diagnosis of FRI is not clear [2,10]. This systematic review summarizes the available evidence on sonication fluid culture and tissue tests including culture, molecular diagnostics and histopathology for the diagnosis of FRI. The main hypothesis, that data focusing on sonication fluid and tissue sampling in FRI is limited, was hereby confirmed.

The first diagnostic test studied in this review was sonication fluid culture. Using low intensity ultrasound, sonication is deployed to dislodge the biofilm from the implant. The sonication fluid is then cultured onto bacterial media for further analysis. In PJI, sonication of the implant and subsequent inoculation of sonication fluid has already proven to be useful [22]. Overall, five studies addressing the diagnostic accuracy of sonication fluid cultures for FRI corresponded to the eligibility criteria and were included in this review. One study by Renz et al. looked into the validation of sonication fluid PCR for the diagnosis of FRI, but compared sonication fluid cultures with tissue cultures as well. No statistically significant difference was found between both methods. [17]. All five studies focusing on sonication fluid culture included tissue cultures in their assessment and reported a high sensitivity for sonication fluid culture [11-15]. Three out of five studies did not assess diagnostic test accuracy separately for FRI patients, but combined results for PJI and FRI [13-15]. A statistically significant difference between sonication fluid culture and tissue culture was reported in three studies, whereby sonication fluid culture showed higher diagnostic accuracy [12, 13, 15]. Unfortunately, only one of these studies assessed these diagnostic tests separately for FRI, providing a customized definition [12] and none of these three studies was sufficiently powered to give a definitive comparison. Indeed, the study by Yano et al. is the only study that included only fracture cases and provided a custom definition for 'osteosynthesis-associated infection'. This study as well as the study by Portillo et al., Holinka et al. and Renz et al. followed the sonication protocol as it was originally described [22]. Regarding tissue samples, poor sampling methods were used in the study by Yano et al., with a requirement of 'more than one peri-implant tissue samples', while current guidelines recommend 3 to 5 or even 6 tissue samples to be taken intraoperatively [22-24]. The incubation of tissue samples in the study by Yano et al. as well as by Portillo et al. was done in accordance to current practice: cultures are generally incubated for 7 days, but there is evidence for extending the incubation period to 14 days in order to

isolate less virulent anaerobic pathogens [24]. Overall, exact data on sampling methods (i.e. sampling location, transport and culture methods) was limited across studies. This is a limitation as high quality, uncontaminated, deep tissue and implant samples are essential to validate the outcome of cultures.

Free planktonic bacteria are considered to be more susceptible to antibiotic therapy than those organized in a biofilm on the implant. Therefore, in order to avoid false-negative culture results, it is generally advised to stop antimicrobial therapy two weeks before sampling [3, 12, 23]. In PJI, sonication fluid cultures were found superior to tissue cultures when patients received antibiotics preoperatively [22]. It seems that in patients who received antibiotics prior to surgery, sonication fluid cultures may be a useful adjunct in the diagnostic algorithm for FRI as well. Furthermore, sonication fluid cultures obtained by inoculation in hemoculture bottles were not at all influenced by previous antibiotic treatment [13]. Important to note is that, for the studies in this review, the sample sizes for the sub-analyses regarding preoperative antibiotics were rather small, with 22 patients in the study by Portillo et al., 39 patients in the study by Yano et al. and 19 patients in the study by Puig-Verdié et al., adding up to a total of only 80 patients who received antibiotics prior to surgery [11-13]. Therefore, it is difficult to make a statement based on these results.

Although there is no strong evidence that sonication is superior to tissue culture in the diagnosis of FRI, it may be a useful adjunct, alongside conventional cultures. There is weak evidence that it may be helpful in patients who have received antibiotics prior to surgery. However, it has to be stated that heterogeneity in study designs, sampling and lab protocols make it difficult to compare results. To be able to make a definitive statement on the role of sonication in the diagnosis of FRI, further research by means of studies on FRI, using standardized protocols and reference criteria, is required.

Molecular diagnostics were the second studied modality in this systematic review. Known clinical applications of PCR are, as adjunct tests, the diagnosis of endocarditis and PJI [2, 25-27]. Overall, it is expected that, due to its high sensitivity, PCR has the potential to detect bacteria despite antibiotic therapy. The additional advantage of PCR is short processing time (<5 h) and the fact that it can be fully automated [26]. On the other hand, interpretation of PCR results in patient management must be done with caution due to potential false positive results [28]. This review identified one study by Omar et al. looking into the diagnostic accuracy of 16S rRNA PCR for the diagnosis of FRI [16]. The authors found 16S rRNA PCR of deep wound swabs inferior to standard tissue cultures. In general, deep tissue swabs are not considered standard of care because they do not sufficiently represent the pathogens in the bone [2, 3, 29]. The other study

by Renz et al. [17] focused on the validation of multiplex PCR on sonication fluid. The performance of sonication fluid PCR for the diagnosis of FRI was comparable to tissue culture tests. Palmer et al. published a study comparing multiplex PCR combined with mass spectrometry and FISH to conventional tissue cultures for the diagnosis of non-unions. This study did not meet our eligibility criteria as it was not a validation study. However, it did provide some interesting preliminary results: multiplex PCR with mass spectrometry identified the cases that were determined by conventional tissue cultures, but this method found additional infected cases as well. All additional cases could be confirmed by 16S rRNA FISH, thereby confirming the high sensitivity of this diagnostic test [30]. With further improvement of the performance, PCR has the potential to complement conventional cultures.

As a final topic, we considered all articles assessing histopathology for the diagnosis of FRI. Histopathology is already an accepted technique for PJI [18]. Three studies focusing on histopathology as a diagnostic test for infected fractures were identified. The study by Simpson et al. provided evidence that histopathology of tissue within and around a fracture non-union can be a useful adjunct to standard microbiological tissue cultures. The authors used the average count of a minimum of one polymorphonuclear neutrophil (PMN) per high power field (HPF) after examination of approximately 10 HPFs as highly suggestive for infection [18]. The study by Chadayammuri et al. demonstrated a lower sensitivity and very low specificity [19]. However, this study has some important limitations. First of all, no criteria or thresholds, i.e. number of PMN's per HPF suggestive for infection, were provided. Secondly, they used 'open bone biopsy and culture' as the reference method, without further specification. It is not clear if this includes histopathology of bone as well. This is an important limitation as cultures can yield false positive or false negative results too. Thirdly, they included swab cultures in their assessment, which is not a standardized technique as described above. Due to the high number of inaccuracies in this study, its outcome cannot be relied upon in clinical practice. The study by Egol et al. was identified by going through reference lists. This study showed poor results for both frozen section as well as permanent section histopathology. Little information was provided on sampling methods and remarkable is that the reference standard for this study was a positive intraoperative tissue culture, which can give false positive or false negative results as well. Therefore, due to these limitations, its outcome cannot be relied upon in clinical practice. Holinka et al. aimed at validating the use of sonication fluid cultures compared to tissue cultures. The authors included histopathology in their assessment as well and reported a sensitivity of 95% and a specificity of 100% [15]. Renz et al. also included histopathology in their study and reported a sensitivity of 74% and specificity of 100% [17]. It should however be noted that the validation of histopathology was not the main objective in these studies

and that Holinka et al. included a majority of patients with prostheses. As previously published, histopathology has already proven its use in PJI [15, 17, 31]. Overall, the implementation of histopathology in the diagnostic algorithm for FRI seems very interesting. The difficulty is that PMN's play an important role in the early phases of fracture healing [32]. After three to four weeks, acute inflammatory cells are less frequent and the presence of larger numbers may indicate infection. The diagnosis of early, acute fracture infections is often less problematic, with discharging wounds and virulent organisms, which are easier to culture [3]. In a recent study of FRI in cases more than four weeks from fracture, published after this search, a bimodal cut-off for the presence of PMN's provided encouraging results in reducing the number of cases in which the diagnosis was uncertain [33].

The limitations of this systematic review are mainly related to the previous absence of a working definition of FRI. It is essential that future evaluations of diagnostic techniques are performed in large numbers of patients and compared against an accepted definition, which includes clear clinical and microbiological criteria [10]. Studies should report the sampling method, the laboratory culture technique and number of samples cultured.

In conclusion, the presented systematic review confirms the hypothesis that scientific evidence on sonication, molecular techniques and histopathology for the diagnosis of FRI is scarce. Sonication has been extensively investigated in PJI, but the few studies on FRI are heterogeneous in design and have applied a variety of reference standards. Although these studies have shown encouraging results for sonication fluid culture, the evidence is of low quality and we cannot conclude that sonication fluid culture is superior to a good standard of tissue culture as recommended for the diagnosis of FRI. Regarding molecular techniques and histopathology, evidence is scarce and based on small studies. Further study and improvement of diagnostic performance is warranted.

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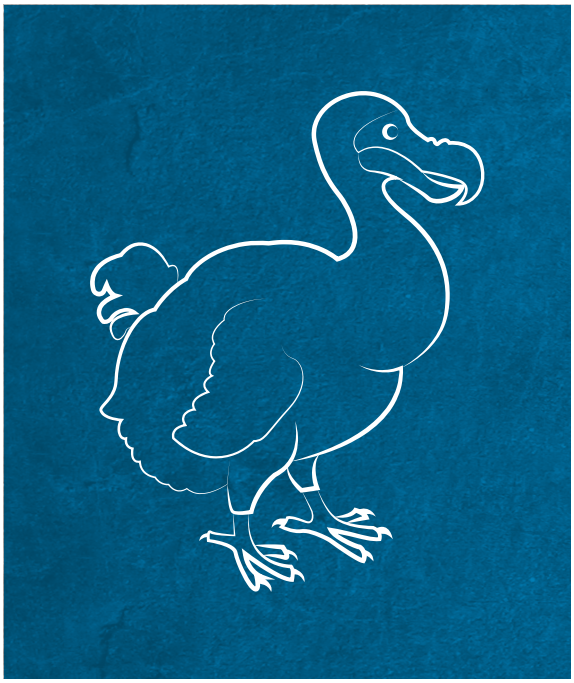

PART V

IMPLEMENTATION AND FUTURE PERSPECTIVES

CHAPTER 11

STAND VAN ZAKEN
Diagnostiek en behandeling van fractuur-gerelateerde infecties

G.A.M. Govaert en M.F. Termaat namens de richtlijnwerkgroep fractuur-gerelateerde infecties

Ingediend

Leden van de richtlijnwerkgroep fractuur-gerelateerde infecties

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SAMENVATTING

- Fractuur-gerelateerde infecties resulteren in ernstige morbiditeit, verlies van kwaliteit van leven en sterk verhoogde zorgkosten. Ondanks het feit dat fractuurchirurgie al ruim anderhalve eeuw wordt toegepast is er vaak onvoldoende aandacht voor een gestructureerde behandeling van deze ernstige complicatie.
- Op verzoek van de Nederlandse Vereniging voor Heelkunde (NVvH) is de richtlijn "diagnostiek en behandeling van patiënten met een fractuur-gerelateerde infectie" ontwikkeld, geschreven voor alle leden van de beroepsgroepen die betrokken zijn bij de zorg voor deze patiëntengroep.
- Dit artikel is een samenvatting van de richtlijn en beoogt aan de hand van een klinische casus een leidraad te geven voor de diagnostiek en behandeling van patiënten met een fractuur-gerelateerde infectie.

Verklarende FRI- woordenlijst

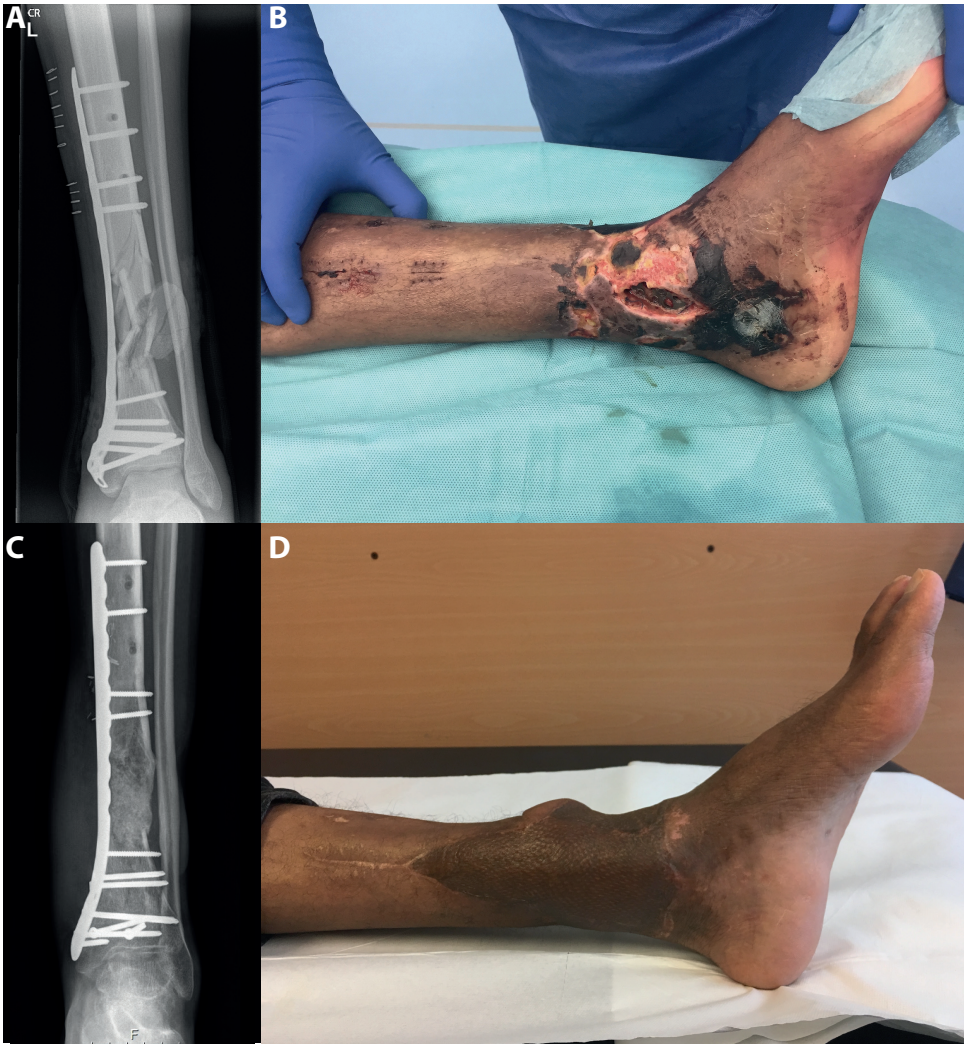
Sinus: een lichaamsholte.
Fistel: een niet-natuurlijk kanaal tussen twee lichaamsholten of tussen een lichaamsholte en de huid.
Involucrum: een laag van nieuwe botvorming aan de buitenkant van bestaand bot. Dit wordt veroorzaakt door het loslaten van periost door onderliggende pusophoping en vervolgens nieuwe botgroei vanuit het periost.
Cloacae: een opening in een involucrum waardoor pus en necrotisch material draineert. Dit kan resulteren in het ontstaan van een fistel.
Sequester: dood botfragment. Dit kan ontstaan doordat tijdens het trauma het periost van een botdeel wordt afgetrokken waardoor het bot onvoldoende bloed krijgt en afsterft of door het proces van toenemende necrose ten gevolge van infectie bij initieel vitaal bot. Een sequestrum is een veelvoorkomende complicatie (sequela) van FRI en kan ook de infectie in stand houden.
Biofilm: een laag micro-organismen ingebed in zelfgeproduceerd slijm en vastgehecht aan een dood oppervlak (bijvoorbeeld een sequester of osteosynthesemateriaal). De micro-organismen in een biofilm verkeren vaak in een stationaire groeifase, hebben een verlaagd metabolisme niveau en zijn relatief ongevoelig voor het afweersysteem van hun gastheer of antibiotica.

INLEIDING

Een 26 jarige man raakt bekneld met zijn linker been waardoor er een Gustillo graad 3B open distale tibiafractuur ontstaat [Figuur 1]. De wond wordt onder narcose gereinigd en gesloten en de fractuur wordt gestabiliseerd. Vanwege een fractuur-gerelateerde infectie (*fracture-related infection,* FRI) met necrose van de huid en blootliggende plaat wordt patiënt 3 weken na trauma overgeplaatst naar ons centrum. Na uitvoerig debridement van de weke delen en verwijdering van sequesters wordt een reconstructie met behulp van een zogenaamde *induced membrane technique* (operatie volgens Masquelet) uitgevoerd. Hierbij wordt het botdefect tijdelijk opgevuld met antibiotica-houdend cement, de mediale plaat gewisseld en het weke delen defect gesloten met een vrije latissimus dorsi lap. Zes weken later volgt de tweede fase waarbij het cement wordt vervangen door spongiosa uit het linker femur. Na 1 jaar is de fractuur geconsolideerd en volledig belastbaar met goede weke delen.

Een postoperatieve infectie na fractuurbehandeling is een ernstige complicatie en leidt vaak tot een langdurig behandeltraject met verlies van kwaliteit van leven en 650% stijging van medische kosten [1, 2]. De incidentie van fracturen bij volwassenen ($\geq$16 jaar) in Nederland is 1291/100.000 inwoners per jaar (data 2012) en één derde van deze patiënten ondergaat hiervoor een operatie [3]. De gerapporteerde incidentie van FRIs varieert, gemiddeld tussen de 1 en 5 %, in sommige gevallen zelfs 45%, en is afhankelijk van de aanwezigheid van risicofactoren zoals diabetes mellitus, roken, open fracturen en contaminatie van de wond [4, 5]. Dit betekent dat in Nederland ieder jaar ongeveer 3000 volwassenen met een FRI gediagnosticeerd worden, dit is één patiënt per iedere twee huisartsenpraktijken en ongeveer 20 patiënten per ziekenhuis.

Op verzoek van de Nederlandse Vereniging voor Heelkunde werd recent de richtlijn "diagnostiek en behandeling van patiënten met een fractuur-gerelateerde infectie" ontwikkeld. Deze richtlijn is geschreven voor alle leden van de beroepsgroepen die betrokken zijn bij de zorg voor patiënten met een FRI (zowel in de eerste, tweede als derde lijn) en omvat alle infecties ontstaan tijdens fractuurbehandeling. Infecties bij gewrichtsprotheses en hematogene botinfecties vallen buiten het bestek van deze richtlijn. Op basis van knelpunt analyses werden onderzoeksvragen opgesteld die vervolgens door middel van systematisch literatuuronderzoek werden getracht te beantwoorden. De richtlijnontwikkeling werd ondersteund door het Kennisinstituut van Medisch Specialisten en gefinancierd uit de Stichting Kwaliteitsgelden Medisch Specialisten.



Figuur 1 A. AP röntgenfoto van het linker onderbeen van patiënt ten tijde van overplaatsing. Er is sprake van een comminutieve distale tibiafractuur welke gestabiliseerd is door middel van een mediale plaatosteosynthese. **B.** Klinische foto van het linker onderbeen ten tijde van overplaatsing. Er is sprake van een fractuur-gerelateerde infectie met uitgebreide necrose van de omliggende weke delen, wonddehiscentie en een blootliggende plaat. **C.** AP röntgenfoto van het linker onderbeen na 1 jaar. Er heeft een reconstructie van het botdefect plaatsgevonden met de zogenaamde *induced membrane technique* (operatie volgens Masquelet) en de mediale plaat is gewisseld. De spongiosaplastiek is geconsolideerd. **D.** Klinische foto van het linker onderbeen na 1 jaar. De weke delen zijn gereconstrueerd met behulp van een vrije Latissimus Dorsi lap welke bedekt is met een huidtransplantaat.

In dit artikel bespreken wij de huidige stand van zaken rondom de diagnostiek en behandeling van fractuur-gerelateerde infecties te aan de hand van bovengenoemde richtlijn.

Definitie

Sinds 2018 worden infecties ten gevolge van fractuurbehandeling aangeduid als "fractuur-gerelateerde infecties" conform de recent gepubliceerde consensus van de *Arbeitsgemeinschaft für Osteosynthesefragen* (AO) en de European Bone and Joint Infection Society (EBJIS) [6]. Deze definitie maakt gebruik van suggestieve en bevestigende criteria [Tabel 1]. Er wordt geen onderscheid gemaakt tussen "oppervlakkige" of "diepe" infecties omdat diagnostiek en behandeling voor beide categorieën gelijk is. In de aanwezigheid van recent geplaatst osteosynthesemateriaal is de kans op een oppervlakkig infectie zeer klein en een diepe infectie moet altijd worden uitgesloten door middel van lege artis afgenomen diepe wondkweken. Het veel gebruikte onderscheid tussen een "vroege" versus "late" FRI is arbitrair en de AO/EBJIS definitie maakt ook hierin geen onderscheid. De richtlijncommissie meent wel dat het relevant is om onderscheid te maken of een infectie vroeg of laat na de initiële operatie wordt herkend en behandeld. Het belangrijkste argument hiervoor is gebaseerd op het concept dat een biofilm enige tijd nodig heeft om te ontwikkelen en in de late fase antibiotica lastiger deze barrière penetreren waardoor de infectie moeilijker te bestrijden is [7]. Daarom wordt in de richtlijn FRI onderscheid gemaakt tussen "vroege" (ontstaan binnen 6 weken na operatie) en "late" (ontstaan meer dan 6 weken na operatie) infecties [8].

DIAGNOSTIEK

Wanneer op grond van lokale en systemische infectieuze symptomen (zoals pijn, erytheem en koorts} de verdenking op een FRI bestaat zal in de regel aanvullend onderzoek worden verricht. De meest gebruikte aanvullende diagnostiek voor FRI bestaat uit het bepalen van serum inflammatie markers, medische beeldvorming en microbiologisch onderzoek. Histologisch onderzoek wordt in Nederland nog nauwelijks toegepast bij het stellen van de diagnose FRI hoewel er recent (na het tot stand komen van deze richtlijn) een studie is verschenen die het gebruik hiervan ondersteunt [9]. Hieronder worden de drie gangbare categorieën aanvullende diagnostiek besproken.

Tabel 1: Diagnostische criteria fractuur-gerelateerde infectie [7]

Bevestigende criteria
- de aanwezigheid van pus - een fistel, een sinus en/of falen van de wond *("wound breakdown")* communicerend met het bot of implantaat - aanwezigheid van fenotypisch identieke pathogene micro-organismen in minimaal 2 diep afgenomen weefselspecimens (of sonificatievloeistof) bevestigd met microbiologisch onderzoek - aanwezigheid van pathoge(e)n(e) micro-organisme(n) in minimaal 1 diep afgenomen weefselspecimen bevestigd met histopathologisch onderzoek
Suggestieve criteria
- lokale en systemische infectieuze symptomen (pijn, erytheem, koorts en/of nieuw ontstane gewrichts effusie) - radiologische kenmerken (loslating van osteosynthesemateriaal, sequestratie, vertraagde fractuurgenezing, aantasting van cortex) - verhoogde ontstekingsparameters - persisterende, toenemende of nieuwe wondlekkage - aanwezigheid van pathogene micro-organisme(n) in minimaal 1 diep afgenomen weefselspecimen bevestigd met microbiologisch onderzoek

Ieder op zichzelf staand bevestigend criterium is genoeg om een fractuur–gerelateerde infectie (FRI) te diagnosticeren. Bij de aanwezigheid van pus, een fistel of falen van de wond hoeft er dus niet ook nog sprake te zijn van positieve kweken (dit is het geval bij de zogenaamde kweek-negatieve FRI, bijvoorbeeld ten tijde van langdurig antibioticagebruik). De aanwezigheid van suggestieve criteria moedigen aan tot het nader onderzoeken van de mogelijke diagnose FRI.

Ontstekingsparameters

Ontstekingsparameters (met name C-Reactive Protein (CRP), bezinking (BSE) en het leukocytengetal) spelen een belangrijke rol in het diagnosticeren en monitoren van uiteenlopende soorten infecties. Hoewel veel behandelaars het CRP als de meest geschikte ontstekingsparameter beschouwen voor het diagnosticeren van een FRI [10] is het bewijs hiervoor zeer gering [11, 12].

Beeldvormende diagnostiek

Het doel van diagnostische beeldvorming bij FRI is drieledig: 1) vaststellen óf er sprake is van deze aandoening, 2) indien aanwezig, de uitgebreidheid hiervan bepalen en 3) specifieke anatomische informatie verkrijgen ten behoeve van de planning van de eventuele operatie (denk hierbij aan de aan– of afwezigheid van sequestra, cloacae en abcessen). In de praktijk worden op dit moment diverse diagnostische beeldvormende strategieën toegepast, afhankelijk van de beschikbaarheid en voorkeur binnen het eigen ziekenhuis [10]. Er is geen optimale diagnostische strategie, alle technieken hebben zowel voor – als nadelen en daarmee ook hun eigen specifieke indicatie [13-15]. Er zijn echter wel duidelijke 1^{e} en 2^{e} keuze technieken te benoemen [Tabel 2] en het is essentieel dat de chirurgische en de beeldvormende specialist gezamenlijk een techniek

Tabel 2. Overzicht beeldvormende diagnostiek bij fractuur-gerelateerde infecties.

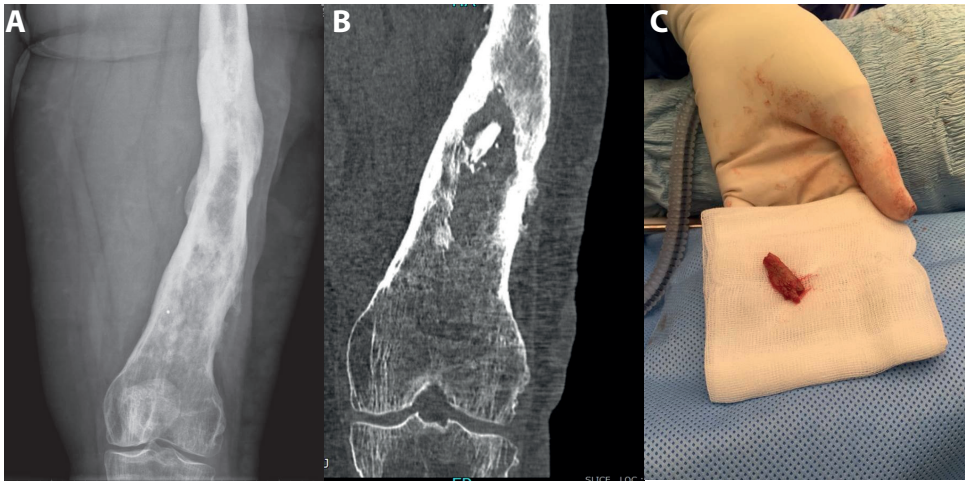
Beeldvormende techniek	Klinische vraagstelling	Voordelen	Nadelen	Sensitiviteit [15]	Specificiteit [15]	Geschatte kosten	Indicatie [13-15]
Röntgenfoto	- beoordeling stand en mate van consolidatie van fractuur - beoordeling stabiliteit van osteosynthese en eventueel materiaal falen	- eenvoudig, breed beschikbaar onderzoek - lage stralingsbelasting	- stralenbelasting - niet goed bruikbaar voor het diagnosticeren van FRI (tekenen van infectie zoals lucentie rondom schroeven en periostale reacties pas in laat stadium zichtbaar)	onbekend	onbekend	50€	- wordt in het algemeen standaard verricht bij patiënten bij wie de diagnose FRI wordt overwogen
CT-scan	- zelfde als bij röntgenfoto	- beter onderscheidend vermogen ten opzichte van röntgenfoto - weinig verstoring van metaal artefacten (mits *iterative metal artefact reduction technique* toegepast) - kan verricht worden met intraveneus contrast (CTA) indien afbeelding van vaatstelsel gewenst (bv voor planning van vrije lap door plastisch chirurg)	- stralenbelasting - niet goed bruikbaar voor het diagnosticeren van FRI	0,47	0,60	250€	- eerste keus onderzoek voor beoordeling stand van de fractuur, de consolidatie en de positie en integriteit van het osteosynthesemateriaal
MRI	- betrokkenheid van de weke delen en afbeelding van ossale anatomie zoals (subcorticale) abcessen, sequestra, involucrae, cloacae en fistelgangen	- geen stralenbelasting - kan verricht worden met intraveneus contrast (MRA) indien afbeelding van vaatstelsel gewenst (bv voor planning van vrije lap door plastisch chirurg)	- ondanks MARS (*metal artefact reduction sequence*) protocollen overprojectie van metaalartefacten nabij implantaten mogelijk	0,82 – 1,00	0,43 – 0,60	300€	- eerste keus onderzoek voor beoordeling weke delen en anatomische bijzonderheden

Tabel 2. (vervolg)

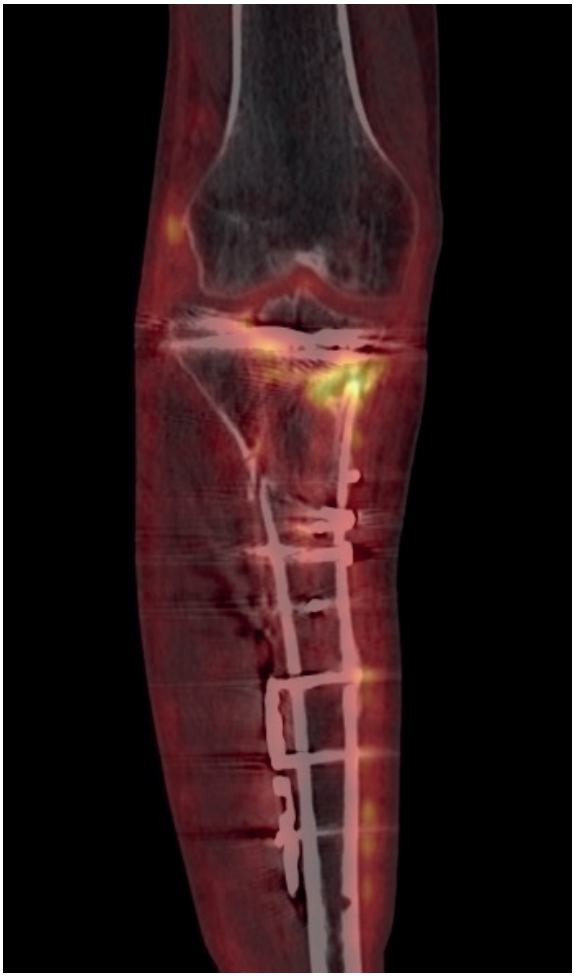
Botscan	- beoordeling vitaliteit van het bot	- vitaliteit bot eenvoudig te beoordelen	- stralenbelasting - iedere vorm van botmetabolisme (bijvoorbeeld bij genezende fractuur) geeft verhoogde opname van tracer	0,89 – 1,00	0 – 0,10	300-400€	- niet geschikt voor het diagnosticeren van FRI
Leukocytenscan + SPECT/CT	- diagnosticeren FRI in perifere skelet	- hoge accuratesse door specifieke leukocyteninfiltratie - hybride imaging levert ook CT beelden (SPECT/CT) - en maakt differentiatie tussen infectie in of buiten het bot mogelijk	- stralenbelasting - bewerkelijke nucleaire labeling en scan procedure (minimaal 2 scan momenten noodzakelijk) - niet geschikt voor diagnosticeren van FRI in centrale skelet	1,00	0,89-0,97	800-1000€	- eerste keus onderzoek voor het diagnosticeren van FRI (gezien hoge diagnostische accuratesse en geen beinvloeding door recente chirurgie) in het perifere skelet
^{18}F-FDG-PET/CT	- diagnosticeren FRI in perifere en centrale skelet	- eenvoudigere diagnostiek (1 scanmoment) - hybride imaging levert ook CT beelden (PET/CT) en maakt differentiatie tussen infectie in of buiten het bot mogelijk - geschikt voor het diagnosticeren van FRI in centrale skelet	- stralenbelasting - lagere diagnostische accuratesse dan leukocytenscan (glucose uptake minder specifiek voor infectie dan leukocyten uptake)	0,86 – 0,94	0,76 – 1,00	1000-1200€	- tweede keus onderzoek voor het diagnosticeren van FRI in het perifere skelet (lagere diagnostische accuratesse maar wel eenvoudigere logistiek) - eerste keus voor het diagnosticeren van FRI in het centrale skelet.

MRI: magnetische resonantie imaging, ^{18}F-FDG-PET: positronemissietomografie met de tracer fluor-18-fluorodeoxyglucose, MRI: magnetische resonantie imaging, MRA: magnetische resonantie imaging angiografie, CT: computertomografie, CTA: computertomografie-angiografie, SPECT: single photon emission computed tomography.

kiezen waar beide mee vertrouwd zijn en deze ook samen beoordelen. Uiteindelijk zijn de bevindingen bij medische beeldvorming een suggestief criterium en geven ze geen 100% zekerheid over het bestaan van de diagnose FRI [6]. In de Figuren 2 en 3 wordt een voorbeeld getoond van de toepassing van respectievelijk CT en FDG-PET/CT bij FRI.



Figuur 2. A: AP röntgenfoto van het linker femur van een 50 jarige man. Patiënt heeft vele jaren geleden een fractuur-gerelateerde infectie van zijn linker femur doorgemaakt en heeft sindsdien last van intermitterende fistels aan de dorsale zijde van zijn bovenbeen. De röntgenfoto laat een afwijkende stand van het femur zien met verdikte cortex en meerdere sclerotische verdichtingen in de mergholte. De fractuur is geconsolideerd en het osteosynthesemateriaal is inmiddels verwijderd. **B:** Coronale CT opname van het distale linker femur toont een intra-medullaire holte met een lengte van 10 centimeter en een los botfragment verdacht voor een sequester. **C.** Peroperatieve klinische foto toont het sequester dat bij exploratie werd aangetroffen. Kweken toonden een infectie met *staphylococcus aureus* aan. Grondig debridement en verwijdering van alle sequestra is essentieel om een recidief FRI te voorkomen.

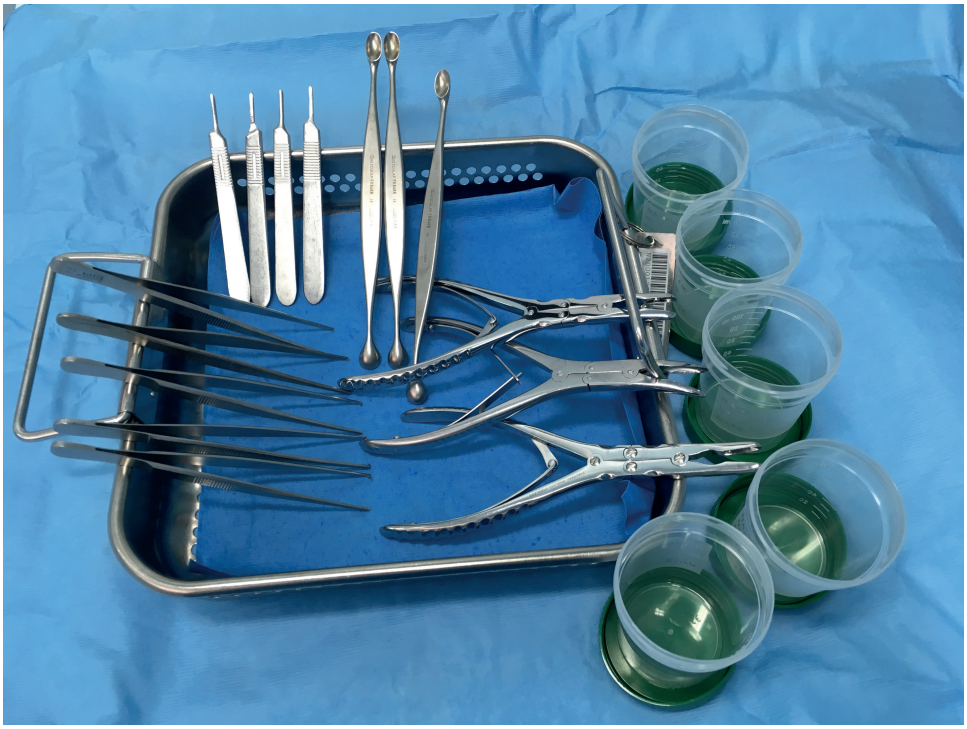


Figuur 3. Een 63 jarige man had vanwege een Schatzker 6 tibiaplateaufractuur links een stabilisatie en plaatosteosynthese ondergaan. Het postoperatieve beloop werd gecompliceerd door een vroege infectie waarvoor hij meerdere malen geopereerd werd maar welke uiteindelijk wel tot rust kwam. Negen maanden later werd het proximale onderbeen spontaan rood en warm, de klachten verdwenen na 2 dagen weer spontaan. Op verdenking van een fractuur gerelateerde infectie werd een FDG-PET/CT verricht welke verhoogde opname FDG uptake liet zien ter plaatse van de mediale plaat. Er werd een chirurgische exploratie verricht waarbij kweken werden afgenomen welke een infectie met een cutibacterium acnes (voorheen proprionibacterium acnes) bevestigde.

Microbiologisch onderzoek

Het microbiologisch onderzoek bij een patiënt met (de verdenking op) een FRI dient zowel een diagnostisch als een therapeutisch doel. Het is hiermee één van de belangrijkste pijlers van het behandelproces en het is daarom van belang om bij het afnemen van de kweken de grootst mogelijke zorgvuldigheid in acht te nemen. Ten eerste moet altijd geprobeerd worden om de verwekker van een FRI te identificeren vóórdat antibiotische therapie gestart wordt. Het blind starten van antibiotica voor een vermeende wondinfectie wordt alleen geadviseerd bij een (dreigende) sepsis. In alle andere gevallen wordt de antibiotica pas toegediend nadat er operatief weefselkweken zijn afgenomen in of nabij het geïnfecteerde bot of osteosynthesemateriaal. Neem voldoende (minimaal 5) kweken af om de kans op fout negatieve uitslagen te minimaliseren [16]. Dit vergemakkelijkt tevens de differentiatie tussen kolonisatie en pathogenen als er laag virulente micro-organismen worden gekweekt. Om contaminatie van deze kweken te voorkomen wordt aangeraden om bij afname van ieder specimen schoon, dat wil zeggen nog ongebruikt, chirurgisch instrumentarium te gebruiken [17]. Het kan handig zijn om hiervoor in uw eigen ziekenhuis een zogenaamd "kweeksetje" te laten samenstellen [Figuur 4]. De diagnose FRI wordt gesteld bij aanwezigheid van minimaal twee morfologisch identieke pathogenen in minimaal twee aparte kweken [6]. Het afnemen van kweken met kweekstokjes kent een fout negatief percentage van ongeveer 30% in vergelijking met weefselkweken en wordt daarom afgeraden [18]. Kweek ook geen fistels en neem geen oppervlakkige kweken of kweken bij negatieve-druk-therapie af. Hierbij wordt voornamelijk de oppervlakkige wondflora en mogelijk niet de diepe verwekker van een FRI gekweekt [19-21]. Dit kan leiden tot verkeerde antibiotica keuzes en daardoor zowel over –als onder behandeling van de eigenlijke verwekker.

Bij het insturen van de kweken dient duidelijk aangegeven te worden dat er sprake is van een (mogelijke) FRI, of er antibiotica werd gebruikt ten tijde van afname van de kweek en of er nog materiaal in situ is. Tot slot is het van belang om de kweken voldoende lang te incuberen (7-14 dagen afhankelijk van het gebruikte medium) om zo ook langzaam groeiende verwekkers te kunnen identificeren.



Figuur 4: Voorbeeld van een chirurgische kweekset. Om contaminatie van de diepe chirurgische weefsel kweken te voorkomen wordt aangeraden om bij afname van ieder specimen schoon, dat wil zeggen nog ongebruikt, chirurgisch instrumentarium te gebruiken. Een eenvoudig "kweeksetje" dat u in uw eigen ziekenhuis kunt laten samenstellen bestaande uit enkele steriele pincetten, scherpe lepels en knabbeltangen volstaat.

BEHANDELING

De optimale behandeling van een FRI leidt tot volledige botgenezing en eradicatie van de infectie met een gesloten huid, binnen een zo kort mogelijke periode en met optimaal behoud van vorm en functie. Vaak is er sprake van een complexe aandoening en afhankelijk van de aard en omvang van de infectie moet er een oplossing worden gezocht voor meerdere problemen. De behandeling bestaat altijd uit twee fases: chirurgie gevolgd door antibiotische therapie.

Chirurgische behandeling

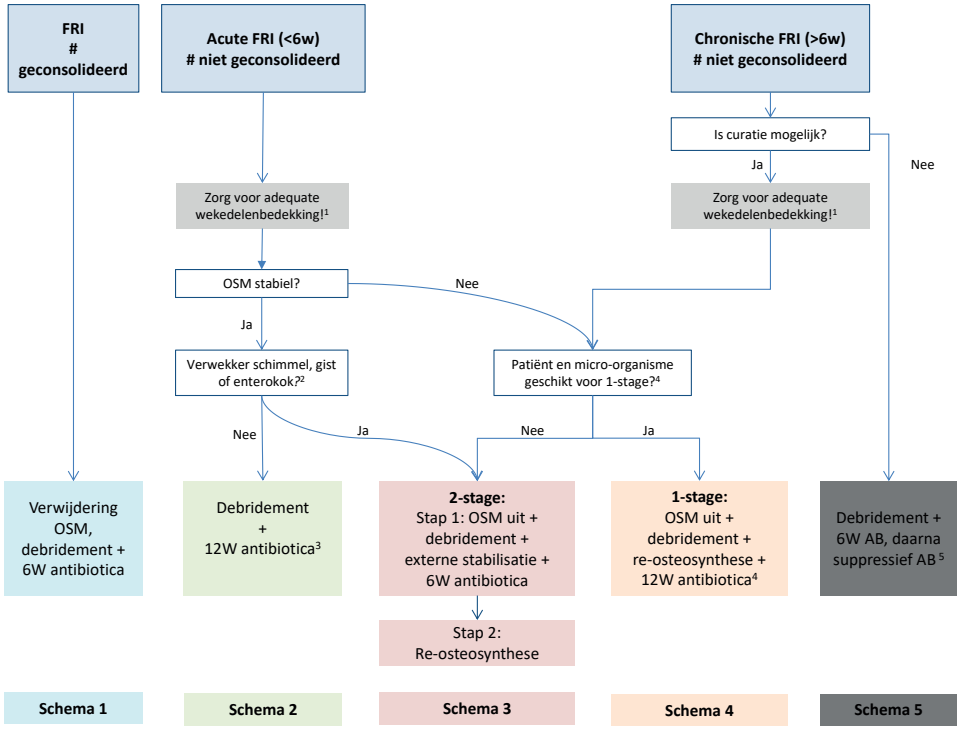
De chirurgische behandeling van een FRI bestaat uit het afnemen van niet-gecontamineerde diepe weefselkweken, uitvoerig debridement met (indien van

toepassing) weke delen bedekking en stabilisatie van de fractuur. Gedurende het debridement worden fisteltrajecten omsneden, wondranden geëxcideerd, pus gedraineerd en necrotisch weefsel, bot en op indicatie het osteosynthesemateriaal verwijderd. Vervolgens wordt het operatiegebied ruim "low-flow" gespoeld met fysiologisch zout.

Op grond van de beschikbare literatuur lijkt het gerechtvaardigd om bij een vroege FRI (binnen 6 weken na de primaire operatie) het materiaal te behouden, mits er sprake is van een stabiele osteosynthese met adequate weke delen bedekking. Bij een late FRI wordt het osteosynthesemateriaal zoveel mogelijk gewisseld. Re-osteosynthese kan zowel in dezelfde operatie ("one-stage") als in een vervolgoperatie ("two-stage") plaatsvinden (Figuur 5). Het gebruik van drains wordt afgeraden omdat dit een 'porte d'entree' voor bacteriën vormt en in de literatuur geen voordeel van het toepassen van drains bij patiënten met FRI bekend is. Adequate weke delen bedekking van een fractuur in combinatie met stabiele osteosynthese is essentieel voor de totstandkoming van consolidatie. Indien de weke delen niet primair gesloten kunnen worden is overleg met de plastisch chirurg, bij voorkeur preoperatief, essentieel. Gezien het ontbreken van bewijs ten aanzien van een voorkeur voor een type weke delen bedekking (fasciocutane -of spierlap) zal er vooral gekozen moeten worden op basis van de grootte en de locatie van het defect en donor-site morbiditeit. Negatieve druktherapie bij patiënten met FRI wordt hooguit aangeraden als tijdelijke bedekking (enkele dagen) bij onvoldoende weke delen bedekking, bijvoorbeeld ter overbrugging naar een huid-spier lap of verwijzing naar een gespecialiseerd centrum. Het langdurig toepassen van VAC therapie leidt, behalve tot verhoging van het aantal ingrepen, ook tot een hoge zogenaamde *bacterial load* en een significante toename van het infectiepercentage en wordt daarom afgeraden [20, 22].

Antimicrobiële behandeling

Er zijn geen goede studies gepubliceerd waarin antibiotische strategieën voor de behandeling van patiënten met een FRI worden onderzocht. Twee gerandomiseerde studies waarin zowel patiënten met prothese infecties als fractuur-gerelateerde infecties zijn geïncludeerd waren underpowered en hadden methodologische beperkingen [23, 24]. Recent zijn de resultaten van de OVIVA trial gepresenteerd (*Scarborough et al.,, EBJIS 2017*), maar nog niet gepubliceerd [25]. In deze trial werden 1054 patiënten met geïnfecteerde gewrichtsprotheses en FRIs gerandomiseerd tijdens de eerste 7 dagen intraveneuze antibiotica. Zodra de verwekker was gedetermineerd werden twee medicamenteuze strategieën vergeleken: direct starten met orale antibiotica (totale behandelduur minimaal 6 weken) versus de eerste 6 weken volmaken met intraveneuze antibiotische therapie.



Figuur 5. Algoritme behandeling fractuur gerelateerde infecties. [1]Met adequate wekedelen bedekking wordt bedoeld: vitale weke delen die spanningsvrij primair gesloten kunnen worden of door middel van transpositie van weke delen vanuit elders in het lichaam. [2] Het betreft geen schimmels, gisten, enterokokken of andere verwekkers waarvoor langdurig toxische antibiotische therapie noodzakelijk is. [3] Behandel stafylokokken met rifampicine-combinatietherapie (bij droge, dichte wond) als (1) de stafylokok rifampicine-gevoelig is en (2) als de verwachting is dat het OSM langdurig in situ zal blijven. Bij gramnegatieven bij voorkeur fluorochinolonen indien gevoelig. [4] Er is geen literatuur over antibiotische therapie bij 1-stage procedures bij FRI. Patiëntgebonden factoren (zoals diabetes, reumatoïde artritis, neutropenie of steroïdengebruik) en het type micro-organismen (langdurig intraveneuze of toxische therapie geïndiceerd zoals vancomycine) spelen een belangrijke rol in de afweging. Het verdient de voorkeur deze beslissing te nemen in overleg met een regionaal expertisecentrum. [5] Duur suppressieve therapie tenminste 12mnd *of* tot kort na verwijdering osteosynthese materiaal.
FRI=fracture-related infection. OSM=osteosynthese materiaal. AB=antibiotica. #= fractuur. W=weken.

Therapie falen binnen 1 jaar na randomisatie was in beide groepen gelijk (in de orale groep 13% versus 14% in de intraveneuze groep). De werkgroep FRI adviseert vooralsnog om de meest gangbare praktijk van initieel twee weken intraveneuze therapie aan te houden. Als we vooruitlopen op publicatie van de resultaten van de OVIVA studie lijkt

het echter mogelijk om eerder dan de nu gebruikelijke 2 weken over te gaan op orale therapie. In afwachting van deze publicatie is het raadzaam om dergelijke beslissingen altijd in multidisciplinair overleg (MDO) te nemen. De duur van behandeling is afhankelijk van het feit of er na chirurgisch debridement nog osteosynthesemateriaal in situ is (12 weken antibiotica) of niet (6 weken antibiotica).

Welke antibiotische therapie wordt gestart hangt af van een aantal factoren, zie ook Figuur 3. Het is echter niet mogelijk om iedere behandelstrategie in een schema te vangen dus individuele uitzonderingen zijn mogelijk. De meeste infecties worden veroorzaakt door stafylokokken en streptokokken en de empirische therapie (de antibiotica die gestart wordt na afname van kweken maar nog voordat de kweekuitslag bekend is) dient hierop gericht te zijn.

FOLLOW-UP

Patiënten dienen minimaal vervolgd te worden totdat het behandeldoel (volledige botgenezing en eradicatie van de infectie met een gesloten huid en met optimaal behoud van vorm en functie) bereikt is. Er is een categorie patiënten waarbij dit doel niet haalbaar is, bijvoorbeeld de inoperabele patiënt die wordt behandeld met suppressieve therapie. Voor deze patiënt zal een passend follow up schema gevolgd moeten worden. Er is geen bewijs in de literatuur dat het routinematig bepalen van ontstekingsparameters gedurende de follow-up of het verrichten van beeldvormende diagnostiek gericht op het vroeger detecteren van een recidief FRI zinvol is. Hetzelfde geldt voor het standaard verwijderen van het osteosynthesemateriaal bij een geconsolideerde fractuur na een doorgemaakte FRI.

TOT SLOT

De behandeling van patiënten met een fractuur-gerelateerde infectie is maatwerk en vraagt om nauwe samenwerking tussen behandelaars in de eerste, tweede en derde lijn. Hier is nog veel winst te behalen en breed ingeburgerde ineffectieve behandelingen (zoals het blind starten van antibiotica bij de verdenking op een postoperatieve wondinfectie na fractuurbehandeling, langdurig antibiotica gebruik zonder chirurgisch therapie of het langdurig toepassen van negatieve druktherapie bij blootliggend osteosynthesemateriaal) dienen vermeden te worden. Het verdient aanbeveling om regionale zorgpaden te ontwikkelen met de mogelijkheid om casuïstiek te overleggen binnen een multidisciplinair overleg en zo nodig patiënten te verwijzen naar regionale expertise centra. In het behandelteam moeten minimaal een

traumachirurg en/of orthopedisch chirurg, een plastisch chirurg en een infectioloog en/of microbioloog vertegenwoordigd zijn. Daarnaast moeten de overige betrokkenen (zoals huisarts, radioloog, nucleair geneeskundige, revalidatiearts, anesthesioloog, apotheker) laagdrempelig geconsulteerd kunnen worden en zo nodig betrokken worden in het overleg. Deze georganiseerde aanpak zal naar verwachting leiden tot betere patiëntenzorg, betere dataregistratie en kosten effectievere behandeltrajecten voor patiënten met een fractuur gerelateerde infectie.

De richtlijn "diagnostiek en behandeling van fractuur gerelateerde infecties" is verkrijgbaar via de website www.richtlijnendatabase.nl.

Leerpunten uit de richtlijn
"diagnostiek en behandeling van fractuur-gerelateerde infecties"

- De diagnose FRI moet altijd overwogen worden indien sprake is van een gestoord of vertraagd genezingsproces van een fractuur.
- Er is géén plaats voor het starten van antibiotica vanuit de huisartsenpraktijk, polikliniek of SEH bij (de verdenking op) een FRI. Dit werkt resistentie in de hand en kan een latere effectieve behandeling vertragen of belemmeren. Essentieel is het (operatief) verkrijgen van diepe weefselkweken vóór start van de antibiotische therapie.
- Het behandeltraject bij een FRI is maatwerk, een multidisciplinaire benadering is hierbij essentieel.
- De chirurgische behandeling van een FRI bestaat uit het afnemen van kweken, uitvoerig debridement, goede weke delen bedekking en (bij niet geconsolideerde fracturen) stabilisatie van de fractuur.
- Neem minimaal 5 diepe weefselkweken ter plaatse van de fractuur en het osteosynthesemateriaal af. Gebruik bij iedere kweekafname schoon chirurgisch instrumentarium, kweek geen fistels, neem geen oppervlakkige wondkweken af en gebruik geen kweekstokjes.
- De antimicrobiële behandeling wordt gestart na afname van de weefselkweken en bestaat uit initieel empirische therapie gericht op de meest waarschijnlijke verwekker(s). De antibiotica wordt vervolgens aangepast op geleide van de kweekuitslagen.
- De duur van behandeling is afhankelijk van het feit of er na chirurgisch debridement nog osteosynthesemateriaal in situ is (12 weken antibiotica) of niet (6 weken antibiotica).
- Overleg laagdrempelig met een regionaal expertise centrum.

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CHAPTER 12

The accuracy of diagnostic Imaging techniques in patients with a suspected Fracture-related Infection (IFI) trial: study protocol for a prospective multicenter cohort study

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Submitted

ABSTRACT

Background. The key for a successful treatment of Fracture Related Infection (FRI) is a prompt and accurate diagnosis. Unfortunately, the optimal diagnostic imaging strategy remains to be established. This study prospectively compares the three commonly used advanced imaging techniques for diagnosing FRI. Primary endpoints are 1) determining the overall diagnostic performances of white blood cell (WBC) scintigraphy, fluorodeoxyglucose positron emission tomography (FDG-PET) and magnetic resonance imaging (MRI) in patients with suspected FRI and 2) establishing the most accurate imaging strategy for diagnosing FRI.

Methods/Design. This study is a non-randomized, partially blinded, prospective cohort study involving two level 1 trauma centers in The Netherlands. All consecutive adult patients who require advanced medical imaging for a suspected FRI are eligible for inclusion. After enrollment and informed consent, patients are scheduled for undergoing all three investigational imaging procedures (WBC scintigraphy, FDG-PET and MRI) within a time frame of 14 days after inclusion. The reference standard for determining the diagnostic accuracy of these imaging modalities will be the result of at least 5 intra-operative sampled microbiology cultures, or, in case of no surgery, the clinical presence or absence of infection at 1-year follow-up. Initially, the results of all three imaging modalities will be available to the treating team. At a later time point, all scans will be centrally reassessed by dedicated medical imaging specialists who are blinded for the identity of the patients and their clinical outcome. The discriminative ability of the imaging modalities will be quantified by several measures of diagnostic accuracy.

Discussion. The IFI trial is designed to resolve the unsolved question on how to utilize advanced medical imaging for diagnosing fracture related infections. The results of this trial will be used to design an evidence-based, feasible and cost-effective diagnostic pathway for patients with suspected fracture-related infections.

BACKGROUND

Fracture-related infection (FRI) [1] is one of the most severe and challenging complications in trauma surgery. The reported incidence generally varies between 1-5%, but may increase up to 45 % in selected patient groups [2, 3]. Known risk factors for the development of FRI in for instance tibial fractures are the previous need for an external fixator (this implies either a severely injured patient or extensive soft tissue damage), time to nailing, open fractures and subsequent Gustilo-Anderson grade [4]. In open fractures, reported risk factors for the development of an FRI are male gender, diabetes mellitus, smoking, a lower extremity fracture, Gustilo-Anderson grade 3 open fracture, contaminated fracture and polytrauma patients [5].

As with most medical conditions, the key for a successful treatment of FRI is a prompt and accurate diagnosis. Particularly a low grade FRI, with an often closed wound and little or no physical inflammatory signs, is not easy to recognize. Commonly requested imaging modalities for bone infections are a conventional X-ray and plain computed tomography (CT) to establish the healing of the fracture and position and integrity of the implants. Subsequently, advanced imaging techniques such as magnetic resonance imaging (MRI), a fluorodeoxyglucose positron emission tomography (FDG-PET) or a white blood cell (WBC) scintigraphy are being used to diagnose FRI. In the last decade the use of hybrid camera systems (Single Photon Emission Computed Tomography (SPECT)-CT, PET-CT or PET-MRI) lead to increased precision of the localisation of the infection and, as a consequence, to higher diagnostic accuracy rates [6-9]. Recently, the European Association of Nuclear Medicine (EANM), endorsed by the European Bone and Join Infection (EBJIS), developed guidelines in which the WBC-scintigraphy with SPECT/CT is regarded to be the most accurate advanced imaging technique for peripheral bone infection. However, this recommendation is based on scarce evidence in the literature and diagnostic studies specifically focussing on FRI are limited and conflicting [7, 10].

To resolve this omission, we designed: The accuracy of diagnostic Imaging techniques in patients with a suspected Fracture-related Infection (IFI) trial.

Primary endpoints:

1. Determining the overall diagnostic performances of WBC scintigraphy + SPECT/CT, FDG-PET/CT and MRI in patients with suspected fracture-related infections.

Secondary endpoints:

1. Determining whether the accuracy of the different imaging modalities is influenced by patient-related factors such as in situ implants (plates, screws and intramedullary nails), recent surgery, open wounds or concomitant antibiotic treatment or comorbidities.
2. Determining which imaging modality provides the most valuable information to the surgeon for planning revision surgery, such as the exact location of the infection, the extent of the infection or the presence of sequestra, cloacae, sinus tracts, intra-cortical or soft tissue abscesses.
3. To assess the quality of life and physical performance of patients with suspected fracture-related infections by using validated patient reported outcome measures.
4. To design an evidence-based, feasible and cost-effective diagnostic pathway for patients with suspected fracture-related infections.

METHODS/DESIGN

Study design

This study is a non-randomized, partially blinded, prospective cohort study involving two level 1 trauma centers in The Netherlands: The University Medical Center Groningen (UMCG) and the University Medical Centre Utrecht (UMCU). The UMCG is the leading and coordinating center of this study.

Patient population

The inclusion and exclusion criteria are presented in Table 1. Patients will be recruited at the trauma surgical or orthopedic outpatient departments, clinical wards and/or the emergency department of the participating hospitals. All consecutive adult patients in the participating centers, who require advanced medical imaging for suspected FRI, are eligible for inclusion. Suspected FRI is defined following the *clinical suggestive* criteria according to the AO/EBJIS definition [1]. These criteria are based on medical history and clinical examination. Patients with clear signs of acute postoperative surgical site infections [11] will be excluded. The same applies to patients who do not need additional diagnostic imaging because the diagnosis of infection can be made without any doubt based on the *confirmative clinical* criteria according to the AO/EBJIS definition. The suggestive and confirmative AO/EBJIS criteria are provided in Table 2.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Age ≥18 years • Suspected FRI according to the suggestive criteria of the AO/EBJIS definition [1]: - local and/or systemic inflammatory signs (e.g. redness and fever) - radiological signs - new onset joint effusion - elevated serum inflammatory markers - persistent, increasing or new-onset wound drainage	• Age < 18 years • Inability to comply with study protocol (for example, due to claustrophobia or expected loss to follow-up < 1 year) • Inability to comply with follow-up (for example, due to language barrier or planned follow up in different hospital) • Known allergies for intravenous contrast or any of the used nuclear tracers • Early (within 30 days) superficial incisional site infections according to the definition of the CDC [11] • No need for advanced imaging techniques because of undisputed FRI according to the confirmative clinical criteria of the AO/EBJIS definition [1] • Pregnant or lactating woman • Uncontrolled diabetes mellitus type 1 or 2

Interventions

After enrollment and informed consent, patients are scheduled for undergoing all three investigational imaging procedures (namely a WBC scintigraphy + SPECT/CT, an FDG-PET/CT and an MRI) within a time frame of 14 days after inclusion in order to determine the most accurate imaging strategy for diagnosing fracture-related infections (Figure 1).

Patient clinical management and follow up

Next to the imaging performed, patients will be treated according to the local standard of care. This includes any additional diagnostic tests, operative treatment, post-operative regimen and postoperative administration of any medication, which will be completely left to the judgement of the treating medical team. The same applies to the decision whether or not to operate based on pre-operative clinical assessment and imaging. The treating surgeon will be aware of the outcome of all regular and research imaging procedures performed in this study prior to the decision making moment whether to operate or not. Included patients who are operated upon the clinical suspicion of an FRI will all have adequate tissue sampling for medical microbiology (MMB). The presence or absence of FRI for this group will be judged based on the outcome of the MMB results.

Table 2. AO/EBJIS suggestive and confirmatory criteria for FRI [1].

Confirmatory criteria
• Presence of a fistula, sinus or wound breakdown (with communication tot he bone or implant). • Phenotypically indistinguishable pathogens identified by culture from at least two separate deep tissue/implant (including sonication-fluid) specimens taken during an operative intervention. • Presence of microorganisms in deep tissue taken during an operative intervention, as confirmed by histopathological examination using specific staining techniques for bacteria or fungi.
Suggestive criteria
• Clinical signs, any one of: - pain (without weight bearing, increasing over time, new onset) - local redness - local swelling - increased local temperature - new onset of joint effusion - fever (single oral temperature measurement of ≥38.3 °C (101 °F)). • Radiological signs, any one of: - Bone lysis (at the fracture site, around the implant) - Implant loosening - Sequestration (occurring over time) - Failure of progression of bone healing (i.e. non-union) - Presence of periosteal bone formation (e.g. at localizations other than the fracture site or in case of a consolidated fracture). • Elevated serum inflammatory markers in case of a secondary rise (after an initial decrease) or a consistent elevation over a period in time, and after exclusion of other infectious foci or inflammatory processes: - Erythrocyte sedimentation rate (ESR) - White blood cell count (WBC) - C-reactive protein (CRP). • Persistent, increasing or new-onset wound drainage, beyond the first few days postoperatively, without solid alternative explanation. • A pathogenic organism identified by culture from a single deep tissue/implant (including sonication-fluid) specimen taken during an operative intervention.

All patients will stay in follow-up according to the current practice and their clinical status will be assessed by an orthopaedic or trauma surgeon at 3, 6 and 12 months (for assessment regarding diagnostic criteria for FRI at follow up see below under *reference standard*). Standard serum inflammation markers (C-reactive proteine (CRP), leucocyte count (LC) and erythrocyte sedimentation rate (ESR)) will be obtained at the time of recruitment and according to the standard of care at follow up (3, 6 and 12 months). When the patient visits the outpatient clinic, he/she will be asked to fill out validated patient reported outcome questionnaires regarding quality of life and physical performance (EQ-5D, SMFA questionnaires). Health-related productivity losses of paid and unpaid work will be quantified by purchasing relevant parts of the institute of

medical technology productivity cost questionnaire (iMTA PCQ questionnaire [12, 13]. The iMTA MCQ questionnaire and the "the Dutch Manual for Costing studies in health care" will be used to calculate the costs of medical consumption during the research period [14-17]. The different time points are summarized in Figure 2.

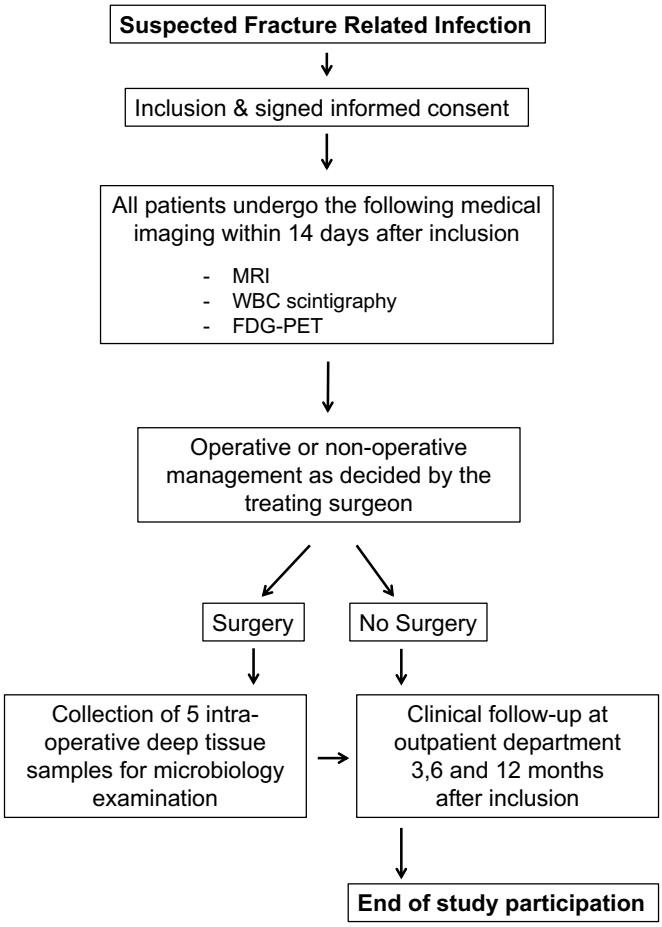

Figure 1. Flowchart of IFI trial.

	STUDY PERIOD							
	Enrolment			Close-out				
TIMEPOINT	$-t_0$	$-t_1$	t_0	t_0	t_1	t_2	t_3	t_4
Time interval	0	< 14 days	if operation	if no operation	3 months	6 months	9 months	1 year
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
INTERVENTIONS:								
MRI		X						
FDG-PET/CT		X						
WBC/AGA scintigraphy + SPECT/CT		X						
ASSESSMENTS:								
Clinical examination	X				X	X	X	X
Labarotory test (CRP, LC and ESR)	X				X	X	X	X
Intra-operative tissue sampling for microbiology			X				.	
Serum inflammation markers (CRP, LC, ESR)	X				X	X	X	X
Questionnaires (PROM and relevant parts of iMTA PCQ)					X	X	X	X

Figure 2. Study assessments IFI trial by time point. Abbreviations: WBC: white blood cell, AGA: antigranulocyte antibody, FDG-PET : fluorodeoxyglucose positron emission tomography, CT: computed tomography, SPECT: single photon emission computed tomography, MRI: Magnetic resonance imaging, CRP: C-reactive protein, LC: leucocyte count, ESR: erythrocyte sedimentation rate, PROM: patient reported outcome, iMTA PCQ: institute of medical technology productivity cost questionnaire.

Assessment of interventional imaging

Initially, all imaging is reported on as usual care in the participating centres dictates. The written reports will be made available through normal routine procedures and available for the treating physician in the electronic medical patient systems. At a later time point, two experienced nuclear medicine physicians (for WBC scintigraphy and FDG-PET) and two experienced radiologists (for MRI), all participating in this trial, will centrally reassess all scans. This is done to obtain uniformity when interpreting the results. All scans will be encoded so that the central reviewers will be blinded for the identity of

the patient and their clinical outcome. Case Report Forms (CRF) are developed to score each scan technique. If the two observers disagree, a third reader will review the images and the final classification will be made in consensus. The scans will finally be classified as "negative for FRI" or "positive for FRI". The reports will also focus on specific anatomic features such as the presence of fistulas, sequesters and soft tissue involvement/ abscesses. In case of surgery, surgeons are requested to fill out a CRF on the operative findings to match these with the anatomic features of the pre-operative scans.

Imaging protocols

Preparation, administration, acquisition and interpretation of all imaging techniques will be performed according to the existing guidelines of the European Association of Nuclear Medicine (EANM) [18, 19] and the European Society of Radiology (ESR). Details are provided in the supplemental materials.

Patient safety

Use of radiopharmaceuticals means exposure to ionizing radiation. Because of the potential hazards of radiation, guidelines for the exposure of healthy volunteers and patients in The Netherlands are specified in "Besluit Stralingsbescherming (BS 2000), artikel 60, Staatsblad 2001, 397" according to the guidelines of the International Commission on Radiological Protection. The extra imaging modality (depending on site specific common practice in collaborating centers) will be the MRI and either the WBC scintigraphy or the FDG-PET. Overall, the additional radiation exposure for a patient participating in this trial will be 4.0 mSv. This complies with ICRP 62, category IIb, which means it is justified in patients.

Reference standard

The reference standard for determining the diagnostic accuracy of the WBC/AGA scintigraphy, the FDG-PET and MRI will be the presence of FRI according to the AO/ EBJIS consensus definition (Table 2). The clinical presence or absence of infection will be judged according these criteria by an experienced trauma or orthopaedic surgeon during follow-up. Cultures will be sampled in a protocolled manner with clean surgical instruments for each sample to avoid cross contamination. At least five samples for microbiology will be obtained. Preferably all antibiotics are discontinued for at least two weeks and antibiotic prophylaxis are withheld until the last sample is obtained [20]. In case of (revision) surgery during follow-up the same sampling protocol will be followed. Intermittent suggestive symptoms such as local pain or redness (with or

without 'treatment' with antibiotics) alone are not regarded confirmative for FRI. They can however prompt the surgeon to order diagnostic tests followed by an operation and tissue sampling, which can subsequently lead to positive MMB results.

Collection of additional medical data

For all patients information concerning patient characteristics, comorbidities, type of implants (plates, screws and intramedullary nails), recent surgery, existence of open wounds, exact location and extent of the infection, presence of sequestra, cloacae, sinus tracts, intra-cortical or soft tissue abscesses, laboratory findings, operation records, microbiology results, NSAID use, nicotine/substance abuse, medication, concomitant antibiotic treatment and the follow up data will be recorded.

Establishing a diagnostic pathway for patients with suspected fracture-related infections

Based on the results of this trial, a diagnostic pathway will be established. In this pathway, the use of the imaging modality or a combination of different imaging modalities with the highest accuracy and feasibility will be described. The costs of the different imaging modalities will be taken into account. For these costs, standard unit prices and rates that are included in the Manual for Cost Analyses, Methods and Standard Prices for Economic Evaluation in Health Care will be used [21].

Sample size and power

Recently, we performed a systematic review about the accuracy of diagnostic imaging modalities for peripheral post-traumatic osteomyelitis (the old terminology for FRI) [7]. Based on the best available evidence over the last 16 years, the sensitivity for WBC scintigraphy ranged from 50 to 100%, and specificity ranged from 40 to 97%. For FDG-PET, this was 83 to 100% and 51% to 100%, and for MRI 82 to 100% and 43 to 60 % respectively. Moreover, studies which combined the WBC scintigraphy with SPECT/CT or the FDG-PET with a CT-scan showed some increase in diagnostic accuracy measures. Overall, our review demonstrated that sensitivity and specificity vary widely between studies, because the literature about fracture-related infections is limited and hampered by small case series, heterogeneous patient populations, different imaging acquisition and interpretations protocols, and quickly evolving (combined) imaging techniques. Another recent publication from our group has shown that the sensitivity and specificity of WBC scintigraphy to diagnose FRI in a large retrospectively analysed patient cohort is 0.86 and 0.98 respectively [22]. A recent retrospective study on the accuracy of FDG-PET/CT reports a sensitivity and specificity of 0.65 and 0.77 respectively [23]. Based on these results we expect a difference of at least 15% in sensitivity and specificity between

the WBC scintigraphy and the FDG-PET. The accuracy of MRI for FRI is reported to be even lower than that of the FDG-PET so this expected difference will also apply for this modality [7].

Sensitivity refers to the percentage of patients who are correctly identified as having the disease, in this case an FRI. With a sample size of N=50 (patients who actually have an FRI) it is possible to detect a statistically significant difference between the sensitivity of WBC scintigraphy (sensitivity of 0.86) and the other diagnostic modalities (maximal expected sensitivity of 0.71): 95% confidence interval (95% CI) around the sensitivity of 0.71 will be 0.58-0.84. Since the sensitivity of WBC scintigraphy of 0.86 is outside this 95% CI, this should be considered statistically significantly relative to the sensitivity of the other diagnostic modalities.

Specificity refers to the percentage of patients who are correctly identified as not having the disease, in this case a FRI. With a sample size of N=120 (patients who do not have an FRI) it is possible to detect a statistically significant difference between the specificity of WBC scintigraphy (specificity of 0.98) and the other diagnostic modalities (maximal expected specificity of 0.83); 95% CI around the specificity of 0.83 will be 0.75-0.91. Since the specificity of WBC scintigraphy of 0.98 is outside this 95% CI, this is statistically significantly different relative to the specificity of the other diagnostic modalities.

In the aforementioned study on the accuracy of WBC-scintigraphy for diagnosing FRI it was established that approximately 30% of the patients who undergo diagnostic imaging for suspected FRI actually have an FRI [22]. The study sample thus needs to contain 170 patients, of whom 50 patients are expected to have an FRI (needed for the sensitivity calculation), and 120 patients are expected not have an FRI (needed for the specificity calculation). To correct for loss-to-follow-up of approximately 20%, a total sample size of N=200 is needed.

Feasibility

We anticipate that we will be able to recruit a sufficient number of patients during the study period. Both contributing hospitals in this multicentre cohort study are level 1 trauma centres with each 50-75 patients suffering from FRI each year (unpublished data on file). We assume that at least half of the eligible patients are willing to participate in this study. This assumption is based on our experience that most trauma patients are eager to comply with additional diagnostic imaging in case of a suspected FRI. This could be due to the fact that they suffer from a long-standing disease and might feel

underdiagnosed. Therefore, we anticipate including 50-60 patients a year in this study. This combined with a follow-up period of 1 year will make it feasible to complete this study in 5 years.

Statistical analysis

Summary statistics and analyses will be provided for all patients who undergo WBC/AGA scintigraphy + SPECT/CT, FDG-PET and MRI. The discriminative ability of the imaging modalities will be quantified by several measures of diagnostic accuracy: sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (PLR and NLR), and the diagnostic odds ratio (DOR). To assess whether a variable (such as type of implants, recent surgery, open wounds, NSAID use or concomitant antibiotic treatment) is predictive of a false imaging test result (false-positive or false-negative versus true-positive or true-negative), multivariable logistic regression analysis will be performed.

Data and safety monitoring board (DSMB) and interim analysis

All research modalities are well-established, common and safe procedures used in clinical practice for this patient population every day. Therefore, the risk of additional adverse events will be minimal. This means that there will be no need to implement a DSMB or interim analysis.

DISCUSSION

Fracture-related infections may seem similar to other orthopedic infections (such as prosthetic joint infections, infected diabetic feet and spondylodiscitis) but there are distinct discrepancies. One of them is that the treatment goal is different: stable fixation and ultimately consolidation of the fracture with the option of implant removal and not, for example, an absolute need for retaining the implant. Similar differences apply for diagnostic imaging. Regenerating bone and soft tissue after surgery and trauma may influence imaging quality and mimic infection. At this moment, no prospective sufficiently powered study has been published on the diagnostic accuracy of medical imaging modalities for FRI [7, 9]. As a result, there are no evidence based diagnostic guidelines or protocols and a variety of diagnostic strategies depending on local availability and local preference of imaging techniques are being used [24, 25]. These random strategies can lead to unnecessary imaging requests and therefore unnecessary delay of treatment and medical costs.

To resolve this omission we designed *The Accuracy of Diagnostic Imaging Techniques in Patients with a Suspected Fracture-related Infection (IFI) Trial*. The results of this trial will be used to design an evidence-based, feasible and cost-effective diagnostic pathway for patients with suspected fracture-related infections.

Declarations

Ethics approval and consent to participate

This study has been approved on August 23rd 2018 by the Medical Ethical Committee of the University Medical Center Groningen (number METc 2018/141) This study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research involving Human Subjects Act.

Availability of data and material

The de-identified datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request after publication of the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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IMAGING PROTOCOLS

1.1 ^{99m}Tc-HMPAO-WBC scintigraphy:

For each WBC-scintigraphy, preparation and labelling of WBC will be performed according to the guidelines for the labelling of leucocytes with ^{99m}Tc-HMPAO of the European Association of Nuclear Medicine.

Acquisition:
Administered activity: according to EANM guidelines.

Early images at 2-4 hrs:
- static images of the affected extremity (anterior, posterior, LPO, RPO), acquisition .time 100 or 200 seconds per view, matrix 256x256, zoom 1.33-1.45.
- SPECT/CT of the extremity (when positive at static images): SPECT: matrix 128x128, zoom 1.0 or 1.23, acquisition counts 20-40 sec/view. Low-dose CT: mAs 30, kV 100, slice thickness 3.0 mm.

Late images at 20-24 hrs:
- static images of the extremity in two directions (anterior/posterioror LPO/RPO, based on early images), acquisition time corrected by time-decay according to time of delayed images, matrix 256x256, zoom 1.33-1.45.

Interpretation:
Visual analysis: of both early and late images with the same count scale or intensity scale:
- increase in WBC uptake over time à infection.
- decrease in WBC uptake over time à inflammation or no infection.
- SPECT/CT for localizing and differentiation between osteomyelitis and soft tissue infection (when uptake increases). The determination of whether there is an infection or not depends on the results of both the early and the late WBC images. With the aid of the SPECT/CT we only intend to determine the exact location of the accumulation. Therefore, the presence or absence of a SPECT/CT in the patient record does not reveal whether there would be an infection or not.

AND

Semi-quantitative analysis: calculation of target-to-background (T/B) ratio in both early and late images. Use contralateral side as background.

- Increase in T/B ratio over time à infection.
- Decrease in T/B ratio over time à inflammation or normal bone marrow uptake, no infection.

1.2 ^{18}F-FDG-PET:

^{18}F-FDG (3 MBq/kg) will be intravenously injected into the patient. Preparation, administration, and acquisition of ^{18}F-FDG-PET will be performed according to the existing guidelines of the European Association of Nuclear Medicine [19]. The scan will be performed 60 +/- 10 minutes after tracer injection. The scanning procedure itself will take approximately 15 minutes.

Acquisition:
Administered activity: according to EANM guidelines.
Image acquisition: according to EANM guidelines/EARL accreditation.

Interpretation:
Visual analysis:
- description of areas with increased uptake: describe intensity of uptake, uptake pattern (homogeneous, heterogeneous), how many foci.
- exact localization of the area(s) with increased uptake

AND

Semi-quantitative analysis:
- calculation of SUVmax, SUVpeak and SUVmean of each area with increased uptake
- calculation of target-to-background ratios (both SUVmax and SUVmean, contralateral side as background) of each area with increased uptake.

1.3 MRI:

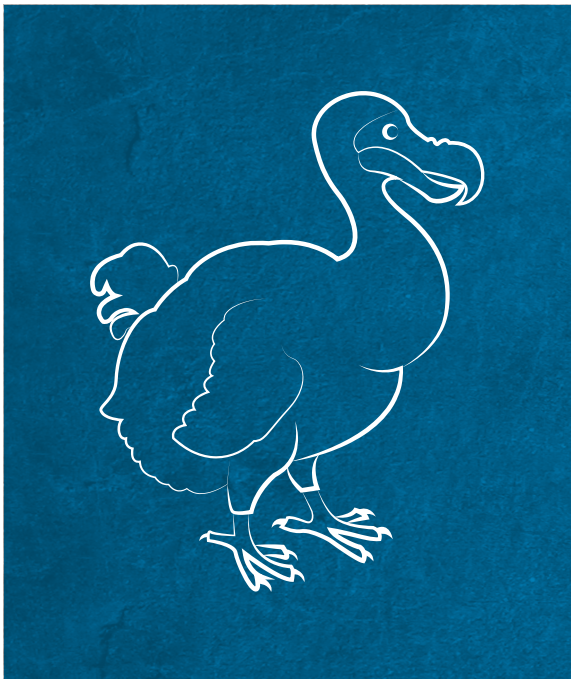
Acquisition:
According to standard MRI acquisition protocol for fracture-related infections:
- 1.5-T or 3.0-T system.
- A surface coil will be used for signal reception.
- Markers will be placed over the area which is clinically suspected of infection or sinus tracts.
- The following sequences will be used: T1-weighted imaging without fat suppression, T2-weighted imaging with STIR fat suppression (no frequency

selective fat saturation techniques), and post-gadolinium fat-suppressed (either (SPIR/SPAIR, or DIXON-based) T1-weighted imaging, all in the axial plane and in one longitudinal direction (either coronal or sagittal) relative to the involved bone.

- All sequences will be performed as turbo spin-echo sequences (no gradient-echo sequences), bandwidth of the excitation pulse and signal read out will be increased, and, depending on local systems and availability, additional metal artefact reduction techniques such as slice Encoding for Metal Artifact Correction (SEMAC or WARP) will be applied *Interpretation:*

Visual analysis:

- Criteria for osteomyelitis:
    - o Low medullary bone marrow signal in a geographic confluent pattern, concordant with abnormal (high) signal at fat-suppressed T2-weighted and post-gadolinium fat-suppressed T1-weighted imaging, is considered to represent osteomyelitis.
    - o Low signal in a subcortical distribution or with a hazy, reticulated pattern at T1-weighted imaging, is not considered to represent osteomyelitis, regardless of appearance at fat-suppressed T2-weighted and post-gadolinium fat-suppressed T1-weighted imaging.
    - o High medullary bone marrow signal at fat-suppressed T2-weighted imaging and post-gadolinium fat-suppressed T1-weighted imaging without corresponding low signal at T1-weighted imaging is considered to represent (reactive) osteitis and not osteomyelitis.
- Associated findings:
    - o Intraosseous, subperiosteal, and soft-tissue abscesses are defined as well-circumscribed areas of focally low signal at T1-weighted imaging with increased signal at fat-suppressed T2-weighted imaging and rim enhancement on gadolinium-enhanced fat-suppressed T1-weighted imaging.
    - o A sequestrum is defined as a structure with low signal at both T1-weighted and fat-suppressed T2-weighted imaging with peripheral enhancement on gadolinium-enhanced fat-suppressed T1-weighted imaging.
    - o An involucrum is defined as a thickened shell of bone around the sequestrum which displays either normal signal or edema.
    - o A cloaca is defined as a focal cortical defect that allows intramedullary pus to drain outward.
    - o A sinus tract is defined as a linear fluid-filled structure extending from bone to the skin surface.
    - o Septic arthritis is defined as joint effusion with synovial thickening.



PART VI

GENERAL DISCUSSION

CHAPTER 13

General Discussion

based on:

Diagnosing fracture-related infections: current concepts and recommendations

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Submitted

ABSTRACT

Fracture-related infection (FRI) is a severe complication after bone injury and can pose a serious diagnostic challenge. Overall, there is a limited amount of scientific evidence regarding diagnostic criteria for FRI. For this reason, the Association for the Study of Internal Fixation (*Arbeitsgemeinschaft für Osteosynthesefragen*, the AO Foundation) and the European Bone and Joint Infection Society (EBJIS) proposed a consensus definition for FRI in order to standardize the diagnostic criteria and improve the quality of patient care and applicability of future studies regarding this condition.

The aim of this paper is to summarize the available evidence and provide recommendations on how to diagnose FRI. For this purpose, the FRI consensus definition will be discussed together with a proposal for an update based on the available evidence on the diagnostic value of clinical parameters, serum inflammatory markers, imaging modalities, tissue and sonication fluid sampling, molecular biology techniques and histopathological examination. Secondly, recommendations on microbiology laboratory operating procedures regarding FRI will be provided.

INTRODUCTION

Fracture-related infection (FRI) is a severe complication after bone injury and can pose a major diagnostic challenge. There is a wide spectrum of clinical presentations of FRI and differentiating them from non-infected causes can be difficult. In the early postoperative period, classical clinical symptoms of infection such as pain, redness, warmth or swelling overlap with features of normal fracture healing. Later, clinically more subtle presentations such as fracture non-union or persistent pain can be attributable to both infectious and non-infected conditions. The complexity and variety of FRI may have, until recently, hindered the establishment of uniform diagnostic criteria. In addition, lack of diagnostic guidance has led to uncertainty in the management and treatment of these patients.

To overcome this challenge, standardized interdisciplinary diagnostic and treatment approaches are mandatory. In contrast to prosthetic joint infection (PJI), protocols tailored to infection in patients after musculoskeletal trauma are scarce. Therefore, many of the surgical and medical treatment concepts applied to FRI have been adopted from PJI treatment algorithms. Both conditions have in common that their diagnosis is a multi-stepped process based on various diagnostic pillars. However, fractures are different compared to arthroplasties. For example, increased bone metabolism due to fracture healing may influence image quality and mimic infection on nuclear imaging and MRI. Therefore it is striking that there is almost no scientific evidence of the predictive value of diagnostic investigations specifically focusing on FRI.

The fact that there was no uniform definition may also have contributed to the scarcity of comparable data on diagnostic strategies for FRI. This shortcoming was confirmed by a recent systematic review, showing that only a minority of randomized controlled trials in fracture care use any kind of standardized definition of FRI [1]. This lack of a universally accepted definition of FRI is similar to the situation for PJI many years ago [2]. The development of uniform criteria for diagnosing PJI have led to an improvement in the diagnostic process of PJI after hip and knee arthroplasty [3, 4]. There is now growing awareness amongst orthopaedic and trauma surgeons that FRI is a unique entity and that also a definition of FRI is required [5]. The need for a uniform definition for FRI is closely related to the need for a uniform diagnostic pathway. In 2015, a survey of 346 Dutch medical practitioners involved in the care for patients with FRI, showed that there was no consensus on the optimal diagnostic strategy for this condition. Also, two thirds of all responders claimed to be unaware of a protocol for the diagnosis and treatment of patients with FRI in their hospital [6].

For all these reasons, the Association for the Study of Internal Fixation (*Arbeitsgemeinschaft für Osteosynthesefragen*, AO Foundation) and the European Bone and Joint Infection Society (EBJIS) recently proposed a consensus definition for FRI in order to standardize the diagnostic criteria and improve the quality of patient care and applicability of future studies regarding this condition [7].

The aim of this paper is to summarize the available evidence and provide recommendations on the diagnosis of FRI. For this purpose, the diagnostic criteria included in the recently published FRI consensus definition will be discussed together with a proposal for an update regarding nuclear imaging modalities and histopathological examination. This update is based on a second consensus meeting including not only the AO Foundation and the EBJIS, but also the Orthopaedic Trauma Association (OTA) and the PRO-implant foundation. Secondly, specific recommendations on microbiology laboratory operating procedures regarding FRI will be provided.

Definition

In 2018, a consensus definition for FRI was published [7]. The development process was comparable to the one used for the new definition on PJI [8]. An international group of experts was involved, representing the AO Foundation and EBJIS as well as prominent orthopaedic trauma hospitals and academic centres with a major interest in FRI. Acknowledging the multidisciplinary aspect of FRI, physicians from different specialties were included. After meticulous exploration of the literature, several video conferences were held. This resulted in a face-to-face consensus meeting where final agreement on the definition of FRI was reached. It was accepted that some features of FRI can be regarded as definitive proof of infection and should be given more weight in the definition. Other less specific features may suggest an infection, but may also be present in patients without infection. This resulted in a set of *confirmatory* criteria (infection definitely present) and *suggestive* criteria (infection possibly present). An updated diagnostic flowchart as proposed by the FRI consensus group will be provided at the end of this paper.

Diagnosis

Diagnosing FRI is a multi-stepped process based on various important diagnostic pillars. This was recognized by the authors of the consensus definition on FRI, who also concluded that solid evidence on which such a definition could be based is scarce [7]. Many of the included criteria were therefore based on expert opinion. In the following sections the diagnostic possibilities for patients with FRI will be described and evaluated based on the evidence available today.

Clinical criteria

Clinical features used to define FRI in the literature were analysed in two recent systematic reviews of 100 and 93 studies, respectively. In the first review, the authors identified definitions used in the scientific literature to describe infection complications after internal fixation of fractures [1]. The second review provided an overview of the available diagnostic criteria, classifications, treatment protocols and patient-related outcome measurements for surgically treated FRI patients between 1990 and 2017 [9]. Both reviews describe a large variety of clinical signs with the only two undisputable definitive criteria, being purulent drainage and wound dehiscence/breakdown. This corresponded to the conclusion of the consensus meeting on FRI: presence of a fistula, sinus or wound breakdown (with communication to the bone or implant) and/or purulent drainage from the wound or presence of pus during surgery are therefore regarded as pathognomonic and are confirmatory clinical signs for the diagnosis FRI [7]. To our knowledge, there are no studies published that report on the predictive value of systemic or local clinical signs of infection for FRI. It was however accepted by the consensus definition group that the clinical signs comprising local redness, swelling, increased local temperature, fever ($\geq$ 38.3 °C), or persistent, increasing or new-onset wound drainage beyond the first few days postoperatively without solid alternative explanation could indicate the presence of an FRI. Therefore, these features are regarded as suggestive clinical signs for FRI. It is important to realize that these suggestive criteria are not pathognomonic and therefore should prompt the treating surgeon or physician to further investigate the possibility of an FRI. It is recommended to document all relevant clinical features meticulously in the patient's medical file, serving as baseline information for future reference as surgical planning and final treatment may be necessary. This includes any local and systemic signs of infection, presence and integrity of (surgical) scars, quality of the soft tissue envelope overlying the suspected site of infection and assessment of the vascular status of the affected limb.

Serum inflammatory markers

The most commonly used serum inflammation markers in orthopedic surgery are leukocyte count (LC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR).

Leukocytes, and more specifically the neutrophils, are the first innate immune cells that are rapidly recruited from the bloodstream to sites of infection and act as major phagocytes. They store large quantities of proteolytic enzymes which can be released when pathogens breach the epithelial barriers. The number of leucocytes and neutrophils can be measured in the blood and therefore they are frequently used as a surveillance tool for (postoperative) infection. Although an increase outside normal

parameters can be an indication of infection, their number will also rise in the presence of other causes of cell damage such as trauma, surgery, sterile inflammation, systemic inflammatory diseases and malignancies [10, 11]. In spinal surgery it is reported that maximum values of LC are seen on day one to three postoperatively and decline to normal values between day four to six [12].

CRP also increases upon various stimuli. Infections, tissue damage, acute coronary syndromes and allergies, for example, can all stimulate pro-inflammatory cytokines. These cytokines, most notably interleukin 6 (IL-6) and to a lesser degree interleukin 1 (IL-1) and tumor necrosis factor-alpha (TNF-α), promote the hepatic synthesis of CRP [13]. Some of the biological functions of CRP are to recognize microbial pathogens and to activate the classical complement pathway. It also activates leucocyte mediated phagocytosis [14]. In fracture patients, CRP levels rise to a maximum on the second day and then concentrations return to normal after approximately 12 days, depending partly on the location of the fracture [15].

Other acute phase proteins (particularly fibrinogen, haptoglobin and ceruloplasmin) and immunoglobulins (mainly IgM) cause a decrease in the negative surface charge of erythrocytes with subsequent increased agglutination and rouleaux formation (stacks of erythrocytes). Infection, amongst other causes, therefore results in an increase in relative weight of erythrocytes compared to serum that is expressed by elevated ESR. Values of ESR peak at day seven to eleven postoperatively and decrease gradually until after week six [16].

Overall, elevation in these three inflammation markers may be seen in trauma patients due to systemic inflammatory response, post-operative or post-trauma tissue damage and surgical and non-surgical infections [11, 17-21]. There is some evidence on the usefulness of measuring CRP, LC and ESR for establishing FRI. In a recent systematic review, the diagnostic value of the serum inflammatory markers CRP, LC and ESR in suspected late onset FRI was assessed [22]. A total of 8280 articles were identified for further evaluation, of which only six were included [23-28]. CRP appeared to be the most useful serum inflammatory marker with a sensitivity ranging between 60.0 and 100% and specificity between 34.3 and 85.7% (cut off values varied between 5.0-10.0 mg/L). Sensitivity of LC ranged from 22.9 to 72.6% and specificity from 73.5 to 85.7% (cut off values ranging from 9.15-10.2 $x10^9$ cells/L). Sensitivity and specificity of ESR ranged from 37.1 to 100% and 59.0 to 85.0%, respectively. Cut-off values ranged between 11.0 – 30.0 mm/h, with two articles using different threshold for men and women. Meta-analysis of the pooled results showed limited diagnostic value of all three markers individually (sensitivity and specificity of 77.0% (95% CI 66.5-85.0%) and 67.9% (95%

CI 38.7-87.6%) for CRP, 51.7% (95% CI 27.2- 12 75.5%) and 67.1% (95% CI 19.3-50.2%) for LC, and 45.1% (95% CI 37.8-52.6%) and 79.3% 13 (95% CI 71.7-85.2%) for ESR. Results of all three markers vary greatly between publications. Another issue that arose when analysing these studies is that different measuring devices, lab protocols and/or thresholds were used. For these reasons, the authors concluded that the analysed serum inflammatory markers – CRP, LC and ESR – seem not suitable to confirm or rule out the presence of FRI.

Therefore, caution when interpreting the results of serum inflammatory markers in daily clinical practice is warranted and they should be regarded only as *suggestive* criteria for FRI, as was published in the consensus definition [7]. Future research, using a clear definition of FRI and standardized lab protocols, will require appreciation of the continuous (as opposed to dichotome) values of serum inflammatory markers to assess their combined value in the diagnosis of FRI.

Medical Imaging

There are three indications to request diagnostic imaging for FRI: 1) to acquire more certainty regarding the presence or absence of FRI, 2) to image the anatomic details of the disease such as its extension, the presence of sequestra, cloacae, sinus tracts and/or subcortical abscesses, for surgical planning, and 3) to establish the degree of fracture healing and implant stability. For these purposes, the clinician has a choice of several radiological and nuclear imaging techniques of which a regular X-Ray, computed tomography (CT), magnetic imaging resonance (MRI), three phase bone scan (BS), fluorodeoxyglucose positron emission tomography (FDG-PET) and white blood cell (WBC) or antigranulocyte antibody (AGA) scintigraphy are the most commonly used modalities. The diagnostic performance of all these imaging modalities is increasing at an almost exponential rate in recent years, due to improvements in technology. This makes it nearly impossible to compare earlier studies with current practice. There is only one study in which the recent literature (from 2000 to 2016) on imaging techniques, specifically for the diagnosis of FRI, was reviewed [29].

Although no good quality study on the diagnostic value of conventional X-ray in FRI exists, this modality is almost always requested first when an infectious complication related to a fracture is suspected [6]. It is easily available, cheap, quickly performed and has a low radiation exposure. Universally accepted suggestive signs of infection are implant loosening, bone lysis, failure of progression of bone-healing (non-union), sequestration and periosteal bone formation [7]. The X-ray also provides baseline information to assess the integrity and stability of any orthopedic implant and is important to assess progress of fracture healing and disease evolution during follow-

up. A CT can also reveal the presence of any sequestra and bone cavities, is an easily performed and readily available imaging technique and it can be combined with an angiography (CTA) in case coverage of a soft tissue defect with a free flap is anticipated. However, its downside is the radiation exposure and the low discriminating capacity for FRI (sensitivity 47%, specificity 60%) [29, 30].

MRI is highly capable of imaging soft tissue pathology. It is also very sensitive for detecting morphologic bony changes which makes it particularly useful in mapping specific surgical details such as the extend of bone and soft tissue involvement, presence of sequestra, cloacae, sinus tracts and/or subcortical abscesses. Another advantage is that an MRI can be combined with angiography (MRA). Unfortunately, distinction between changes due to infection, inflammation and normal tissue healing can be difficult and (despite metal artefact reduction techniques) scattering from metal implants can obscure certain imaging details. Sensitivity and specificity of MRI for detecting FRI are reported to be between 82-100% and 43-60% respectively [29-31].

Nuclear imaging of FRI mainly involves BS, WBC/AGA scintigraphy and/or FDG-PET [6, 32]. An important addition in recent times is the possibility of hybrid imaging (SPECT/CT, PET/CT, PET/MRI) [33]. Sensitivity of BS is high (89-100%), however its specificity is so low (0-10%) that BS is not recommended in the workup of FRI [31, 34, 35]. WBC scintigraphy and AGA scintigraphy are similar scans as both visualize the leukocyte infiltration within the patient. In WBC scintigraphy, the autologous white blood cells of patients are collected, labelled ex vivo and subsequently reinjected. In AGA scintigraphy commercially available labelled monoclonal antibodies against the granulocytes are directly injected and bind in the patient to the leucocytes. It is generally assumed that the accuracy of WBC scintigraphy is higher than AGA scintigraphy because the binding of the labelled WBCs is more specific; however this has not been confirmed in a comparative study for FRI. Sensitivity and specificity of WBC and/or AGA scintigraphy + SPECT/CT for diagnosing FRI is reported to be 79-100% and 89-97% respectively [36-38]. A major advantage of WBC scintigraphy is that its accuracy is not influenced by recent surgery [38]. A disadvantage of this technique is that it is laborious and time consuming as three scans in 24 hours are required and WBC (not AGA) scintigraphy involves manual labelling processes [36]. Furthermore, it is less accurate in the axial skeleton [32, 39]. FDG-PET is slightly less accurate compared to WBC scintigraphy but still suitable for diagnosing FRI, particularly in combination with hybrid imaging (FDG-PET/CT). This technique is based on the fact that in infectious diseases activated leukocytes, monocytes, lymphocytes, macrophages and giant cells all use glucose as their energy source and the accumulation of the ex vivo labelled glucose analogue fluorodeoxyglucose (^{18}F-FDG) can be visualized with a PET camera. The major advantage

of PET above SPECT is a higher spatial resolution (3-4 mm versus 8 mm) and the fact that quantification possibilities are better with PET. Another advantage of FDG-PET is that it only requires one single scan and can also be performed for suspected truncal infections [32]. An FDG-PET should not be utilized for detecting FRI within 1 month after surgery [40]. Sensitivity and specificity of FDG-PET/CT for detecting FRI is between 65 – 94 % and 76 – 100% respectively [40-44]. In Figure 1, a clinical case scenario is provided utilising a WBC-scintigraphy + SPECT/CT to support the diagnosis of FRI of the distal tibia. Figure 2 and Figure 3 show examples of CT and MRI findings in FRI, respectively.

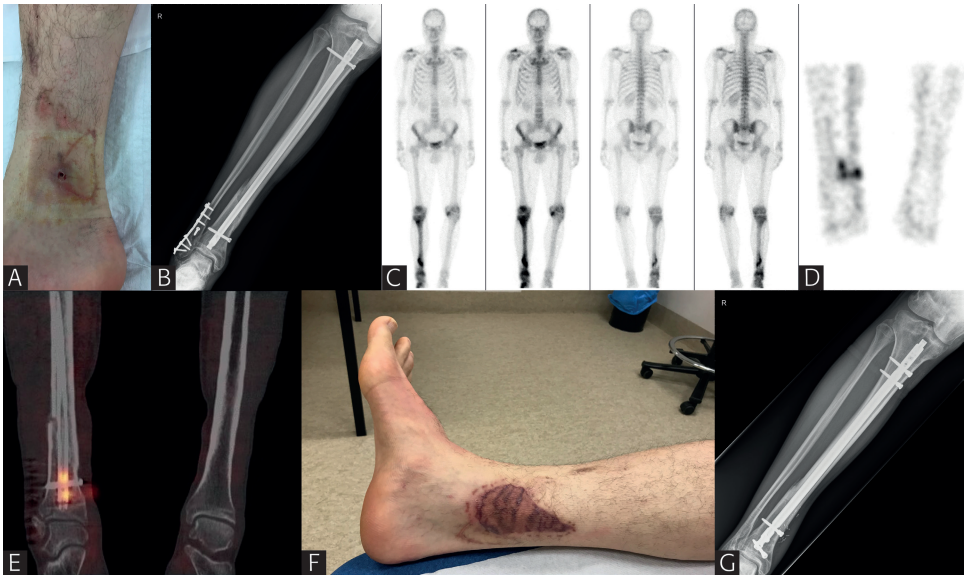


Figure 1. Clinical case scenario (diagnosis made with WBC scintigraphy + SPECT/CT). **A** Clinical image of a 48 year-old male presenting with a fracture-related infection caused by *Enterobacter cloacae* after tibial nailing and fibular osteosynthesis. A draining fistula is *in situ*. **B** Preoperative plain anteroposterior radiograph showing a tibial and fibular nonunion, four months after initial fracture fixation. **C** Preoperative whole body ^{99m}Tc-MDP-scan displaying diffuse bone remodelling in the right knee, lower leg and foot with an increase of bone remodelling surrounding the tibial fracture. **D** Preoperative white blood cell scintigraphy with increased tracer uptake at the medial distal side of the right lower leg. **E** Preoperative SPECT/CT showing a focal increase in tracer uptake at the cutaneous wound, extending towards the distal part of the intramedullary nail and locking screw. **F** One-year postoperative clinical image after antibiotic treatment and a two-stage exchange with a gentamicin-coated tibial nail and coverage of the soft tissue defect by a free muscle flap. **G** One-year postoperative, plain anteroposterior, radiograph showing healing of the tibial and fibular fractures.

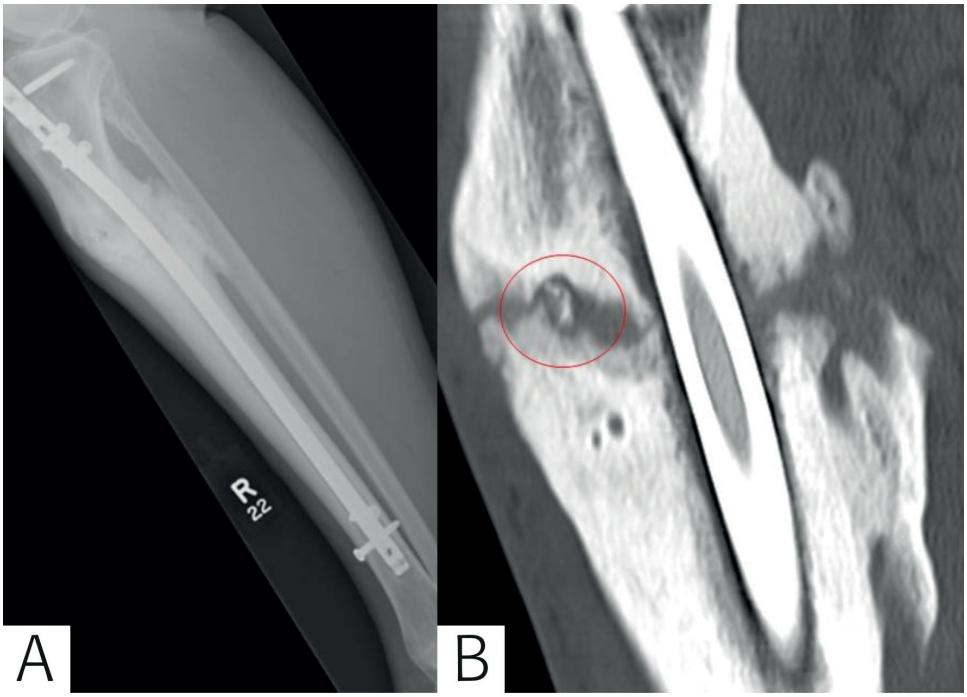


Figure 2. Example of an lateral X-ray (A) and sagittal CT-scan image (B) showing a small sequestrum in an infected non-union which was initially missed on plain x-ray. The patient had already had an exchange nail, without removing the sequestrum and got an immediate recurrence of the FRI.

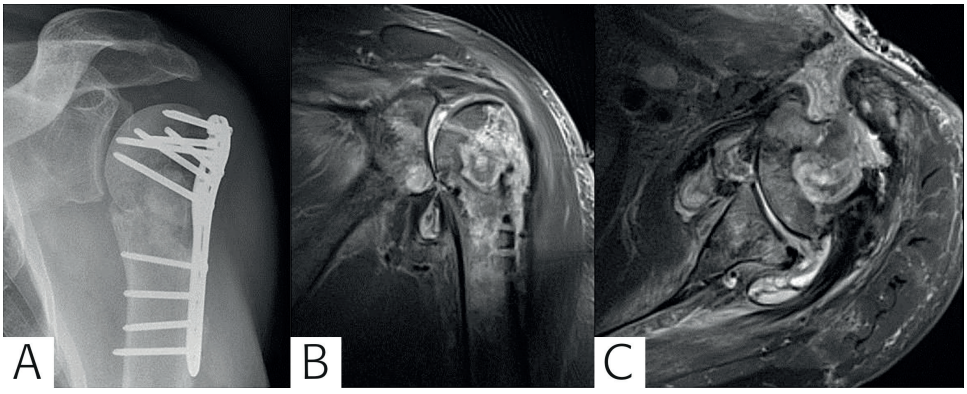


Figure 3. Example of an x-ray (A) and T2-STIR MRI images (B coronal view, C sagittal view) performed in an patient with an infected proximal humerus. The MRI demonstrated the extent of the infection with a skin defect, fluid/pus in the proximal humerus with surrounding oedema, a sinus tract and the involvement of the adjacent joint with possible involvement of the glenoid.

In conclusion, with respect to medical imaging in FRI, every option has its advantages and disadvantages and currently there is no evidence to suggest that one technique is superior over another. The imaging modality of choice depends on local availability, questions to be answered and experience with each technique by the medical specialists involved [6, 32]. Therefore, at this moment, radiological signs can only be regarded as suggestive criteria for FRI [1]. Although nuclear imaging has a higher diagnostic accuracy, it is still not a conclusive test to establish the presence of FRI; therefore it can only be included in the FRI Consensus Definition as a suggestive criterion of FRI.

Microbiology

The culture of phenotypically indistinguishable pathogens from at least two separate deep tissue/implant specimens is considered a confirmative criterion of FRI [7]. In addition, the antibiotic susceptibility of the identified pathogens will guide the choice of antimicrobial treatment. Organisms causing chronic/late-onset infections around implants are often skin commensals and therefore similar to those that can contaminate culture specimens during sampling or handling in the microbiology department. Organisms can also be present in small numbers or may be in slow growth mode in a biofilm. Because false positive or false negative results can lead to erroneous treatment decisions, sampling and culturing techniques should be meticulous.

Surgical sampling protocols have been previously validated for PJI [45] and then applied to FRI [46, 47]. All pre-operative antibiotics should, where possible, be avoided for a period of at least two weeks. Although small [48, 49] and retrospective [50] PJI related studies showed that a a single dose of an antibiotic prior to skin incision makes no difference to the sensitivity of samples, there is a risk that growth of organisms in the laboratory could be inhibited in the presence of antibiotics [51, 52]. It may therefore be more beneficial to administer antibiotics immediately *after* sampling in suspected infection. It is however important to ensure high antibiotic levels prior to a new implant being inserted. A minimum of three but preferably five or more deep tissue or fluid samples should be collected [7, 45, 53, 54], ideally from the implant-bone interface at the site of perceived infection. To avoid cross-contamination it is recommended to minimize manipulation of the target area during this procedure ('no-touch-technique') and to use separate, unused surgical instruments for each sample obtained. A simple sampling surgical instrument set can be assembled for this purpose (Figure 4). Superficial or skin samples should be avoided as these will grow colonizing organisms with no predictive value for the causative pathogen of FRI. The same applies for samples from sinus tracts [55]. Swabs should not be used due to their low yield compared to tissue cultures [56]. After sampling, all specimen containers should be clearly marked

and all relevant details regarding the FRI case are noted on the request form to ensure the samples are processed appropriately. Applying this set of principles regarding tissue sampling techniques has shown to significantly increase microbiological identification with more certainty of causative pathogens for FRI [47]. Finally, the operation note should be clear as to whether pus was found, the extent of infection and whether all infected/non-viable tissue and existing implants were removed or retained. This will enable an appropriate long-term antibiotic plan to be made in due course.

In addition to a meticulous surgical sampling technique, microbiology laboratory operating procedures for processing samples from FRI should be optimised. The relevant stages are as follows: 1) recognizing that these are deep implant-related samples and therefore processing each sample separately (no pooling of specimens), 2) considering methods to disrupt potential biofilm, 3) culturing using appropriate enrichment media for sufficient duration and 4) full identification and a broad antibiogram on each organism to facilitate differentiation of strains and to allow several options for antimicrobial treatment (prolonged course of antimicrobials may be needed and intolerance or hypersensitivity are common).

Figure 4. Example of a surgical instrument set to obtain non-contaminated tissue samples for microbiology and histology. This set can easily be assembled in any hospital and allows the surgeon to use clean, unused instruments for each specimen.

Methods to facilitate biofilm disruption of tissue samples include vortexing with sterile glass beads, possibly with a bead mill or vortexing alone [57, 58]. The role of dithiothreitol or other chemical methods for biofilm disruption is still unclear and needs further evaluation [59]. As organisms can exist in slow growth mode and small numbers, enrichment broth cultures are essential [60]. In sub-acute or chronic infections, plate cultures are not necessary and have low sensitivity. Enrichment broths can be sub-cultured when cloudy or after a defined period of time (e.g. five days). However some organisms take longer to grow and require a later subculture [61]. It should be noted, that multiple sub-culturing stages increase the risk of contamination. Several laboratories associated with bone infection units have therefore moved over to using automated sampling methods to avoid this problem [62, 63]. Using automated methods, cultures that contain pathogens are usually positive by day three and most are positive by day five. In order to culture the slower growing organisms, cultures should continue for around ten days. When cultures are positive, all isolates should be worked up with standard laboratory identification methods and an extended antibiogram including relevant bioavailable and biofilm active antibiotics should be obtained.

Sonication of hard materials can be considered (e.g. plates, nails, cortical bone) but each component only represents one sample. A semi-quantitative cutoff point for the number of colony forming units appears to help differentiate infection from contamination, in PJI [64]. However, as fracture fixation components may not be removed until an hour or so after the start of the surgery and surgical sites get contaminated with organisms by the end of surgery, these results need to be interpreted in context with other findings [65]. In PJI, it is reported that sonication fluid culture is more sensitive than tissue culture when antimicrobial agents were discontinued within 14 days before surgery (75% vs. 45%, $P<0.001$) [64]. The available evidence on sonication fluid sampling and tissue tests (molecular diagnostics and histopathology) for the diagnosis of FRI was analysed in a recent systematic review [66]. Out of 2624 studies, five [67-71] fulfilled the predefined inclusion criteria for sonication fluid culture. This review showed that for FRI there is evidence that sonication fluid culture may be a useful adjunct to conventional tissue culture, but there is so far no evidence that it is superior to tissue culture. Overall, studies had variable 'gold standard' definition criteria for comparison and poorly reported culture methods. The authors concluded that scientific evidence on the accuracy of sonication fluid culture for diagnosing FRI is scarce.

In conclusion, a strict and clear protocol for tissue sampling and laboratory methods for FRI should be adhered to in order to optimise diagnosis, management and long-term outcome. Although sonication seems a useful adjunct to conventional tissue culture, its real added value in the diagnostic process of FRI still needs to be established.

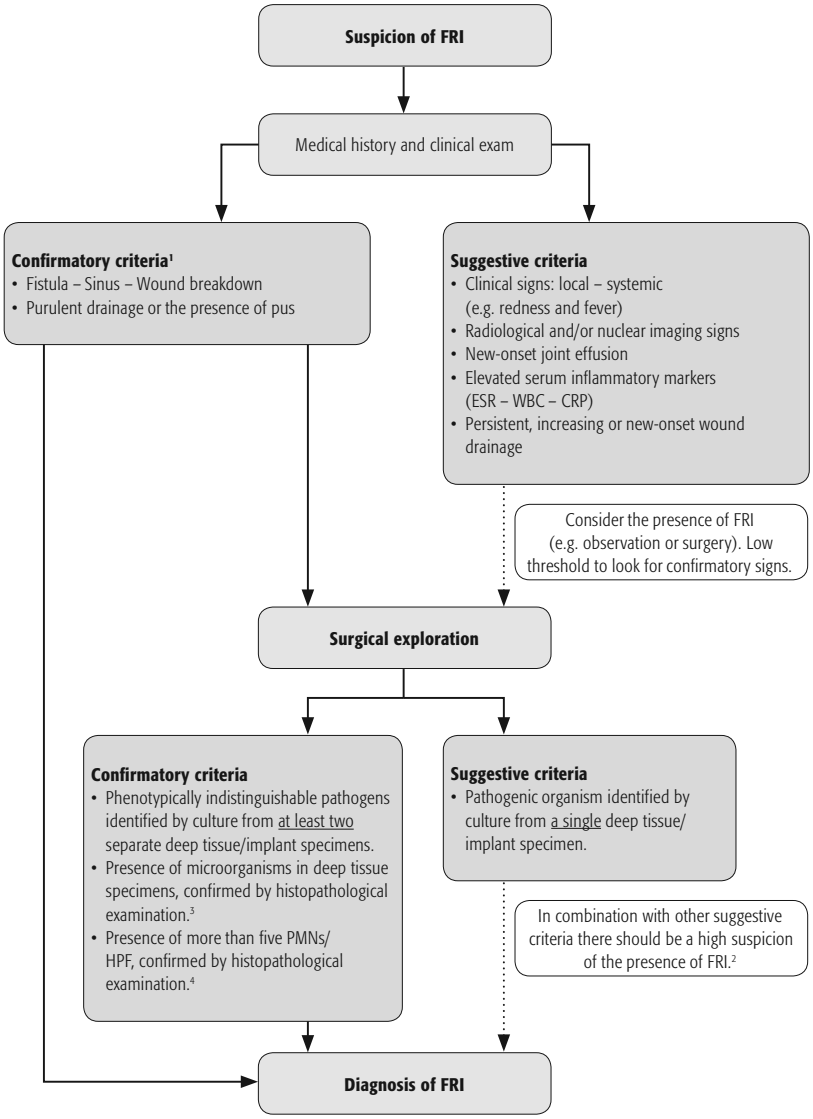
Molecular biology

Polymerase chain reaction (PCR) is a technique that can be used to amplify bacterial DNA. In the past, the amplified PCR products were revealed by electrophoresis in an agarose gel (gel-based PCR). Over the last few years, most gel-based PCR assays have been replaced by real-time PCR. Real-time PCR has the advantage over conventional PCR of speed, as well as being less prone to cross-contamination because it is performed in a closed system. It is a non-cultural identification method that requires a set of primers specific for the targeted bacteria and that is considered to be particularly useful for difficult-to-culture pathogens [72]. It is reported that molecular techniques performed on tissue, synovial or sonication fluid can confer valuable additional information in PJI [73]. Regarding FRI the evidence is less clear [74]. In the aforementioned systematic review also the diagnostic value of PCR techniques for FRI was studied [66]. Two studies were included [24, 75]. The first study reported that 16S rRNA PCR of deep wound swabs are inferior to standard tissue cultures [24]. Unfortunately, this observation is of limited value as deep tissue swabs are already not standard of care due to the fact that they do not sufficiently represent the pathogens in the bone [56]. The other study focused on the validation of multiplex PCR on sonication fluid [75]. It was found that the performance of sonication fluid PCR for the diagnosis of FRI was comparable to tissue culture tests.

In conclusion, evidence on the diagnostic value of molecular techniques for FRI is scarce and based on small studies. Its benefit for diagnosing FRI has not yet been established and further research and improvement of diagnostic performance is warranted.

Histopathology

In PJI, the presence of > 5 polymorph neutrophils per high-power field (PMN/HPF) in 5 high-power fields observed from histologic analysis of periprosthetic tissue, at 400 x magnification, is considered to be an important intra-operative criterion for PJI [4, 76]. In the FRI consensus definition, the presence of visible microorganisms in deep tissue specimens by using specific staining techniques for bacteria and fungi, is regarded a confirmatory sign of FRI [7]. In contrast to the PJI definition, the FRI consensus definition does not yet include the presence of an acute inflammatory cell infiltrate on histopathological examination (e.g. PMN count). At the time of the consensus process, the available literature was extremely poor and no conclusive recommendations could be made, with regard to FRI. The evidence on histopathological examination of tissue specimens for FRI was also investigated in the aforementioned review [66]. It indeed showed to be an underdeveloped area and only three small studies met the pre-defined inclusion criteria [77-79]. The authors of the review reported that scientific evidence on the diagnostic value of histologic examination for FRI is scarce and based on small



[1] In cases of purulent drainage or fistula/sinus/wound breakdown, the presence of pathogens identified by culture is not an absolute requirement (e.g. in the case of chronic antibiotic suppression).
[2] If the positive culture is from sonication fluid, it is highly likely that FRI is present. This is especially true when virulent bacteria (i.e. *Staphylococcus aureus*) are present.
[3] The presence of microorganisms is confirmed by using specific staining techniques for bacteria and fungi.
[4] The presence of an average of more than five PMNs/HPF on histopathological examination should only be considered diagnostic of FRI in chronic/late-onset cases (e.g. fracture nonunion).
ESR: erythrocyte sedimentation rate, WBC: white blood cell count, CRP: C-reactive protein, PMN(s): polymorphonuclear neutrophil(s), HPF: high-power field.

Figure 5. Descriptive flow chart of the diagnostic criteria of FRI. Adapted (with permission) from Metsemakers et al, Injury 2018 [7].

series. Recently however, a study on the value of quantitative histology on diagnosing chronic/late-onset FRI (i.e. unhealed fractures, more than 2 months from injury) was published [80]. In this study, a novel bimodal approach was used to confirm or exclude infection. The complete absence of PMNs has a very high correlation with aseptic non-union (specificity 98%, PPV 98%). On the other hand, the presence of >5 PMN/HPF was always associated with infection specificity 100%; PPV 100%). The combination of clinical signs, ≥2 microbiological cultures and bimodal histological analysis (absent NPs versus >5 PMNs/HPF) improved diagnostic accuracy in up to 96.8% of cases. The authors of this study recommend that these histological criteria can be considered diagnostic of infection in chronic/late-onset FRI (e.g. fracture non-union) and should therefore now be added as confirmative criteria to the FRI Consensus Definition.

In conclusion, the histologically confirmed presence of microorganisms by specific staining techniques on deep tissue specimens is a confirmative sign of FRI [1]. The value of histopathological criteria related to acute inflammatory cell infiltrates (absent PMNs versus >5 PMNs/HPF) is now also established for chronic/late-onset cases (i.e. fracture non-union) and should therefore be included in the FRI consensus definition as a confirmative criterion.

CONCLUSION

Overall, there is limited scientific evidence regarding diagnostic criteria for FRI. With respect to the diagnostic accuracy of serum inflammatory markers, imaging modalities, tissue and sonication fluid sampling, molecular biology and histopathology for FRI, only a small number of studies are available. Validation studies on the value of clinical parameters for diagnosing this condition are non-existent. This lack of scientific evidence precludes the development of a diagnostic pathway that is solely based on sound evidence. The recently published FRI consensus definition seems an adequate start and offers clinicians the opportunity to standardize clinical reports and improve the quality of published literature. It also should lead to a standardized clinical approach towards the diagnostic workup of patients with (suspected) FRI. Apart from the established criteria, there is growing evidence that nuclear medicine imaging and histopathology should play a role in this diagnostic process. During a second consensus meeting in 2018 – including not only experts from the AO Foundation, the EBJIS, but also from the Orthopaedic Trauma Association (OTA) and the PRO-Implant foundation – it was therefore decided that these two criteria will also be included in the FRI consensus definition. Figure 5 shows an update on the current diagnostic criteria. Within the short period since publication, the consensus definition of FRI has already been applied in two

further clinical studies [80, 81]. The continued adoption and evaluation of this definition in further clinical studies will allow validation of the definition and improve the quality of comparative outcome studies in the future.

Recommendations

- The diagnosis of FRI should always be considered in case of impaired fracture healing.
- The presence of confirmative signs of FRI should prompt the treating, multidisciplinary, medical team to proceed with developing a treatment strategy.
- The presence of suggestive signs of FRI should prompt the treating, multidisciplinary, medical team to further investigate the probability of an FRI.
- The only confirmative clinical signs of FRI are the presence of a fistula, sinus or wound breakdown and/or purulent drainage from the wound or presence of pus during surgery.
- Caution when interpreting the results of serum inflammatory markers in FRI is warranted as their predictive value is low.
- The imaging modality of choice depends on the local availability of the technique and the questions to be answered. Nuclear imaging (FDG-PET/CT or white blood cell scintigraphy + SPECT/CT) is more accurate than MRI for detecting FRI but MRI is better in visualizing surgical relevant details. Apart from radiological signs also nuclear medicine signs should therefore be included in the definition.
- As evidence on histopathology is accumulating it seems appropriate to include it in the diagnostic pathway of FRI for chronic/late-onset cases (e.g. non-union).
- Applying a strict and clear protocol for tissue sampling and microbiology culturing for FRI is essential.

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ADDENDUM

ENGLISH SUMMARY

It is difficult to treat a disease that has not been properly diagnosed. One of the challenges of orthopaedic trauma care is that the most accurate and cost-effective diagnostic pathway for diagnosing a fracture-related infection (FRI) has not yet been established. This thesis aims to improve the diagnostic process for FRI by analysing the diagnostic modalities available to date and implementing the outcome in a Dutch guideline and future research. This summary presents an overview of the work that has led to this thesis.

Part I: The problem

In Chapter 1 the burden of fracture-related infections is introduced, the recent AO/EBJIS consensus definition on FRIs is discussed, and an outline of this thesis is presented.

Chapter 2 reports on the results of an online inventory diagnostic survey. There were 346 responders (medical specialists and registrars in orthopaedic and trauma surgery, musculoskeletal (MSK) radiology and nuclear medicine), all involved in the care of patients with FRI. The conclusion of this study was that there is currently no agreement on the optimal diagnostic strategies to diagnose or rule out posttraumatic osteomyelitis (later renamed FRI). Requested medical imaging varies greatly between specialities, and none of the serum inflammatory markers was regarded as very specific for diagnosing FRI. However, for most responders CRP was thought to be the most useful laboratory test for diagnosing FRI. Availability of and awareness towards local protocols to diagnose and treat PTO was poor. The results of this study support the need for future prospective clinical trials on optimal diagnostic strategies for FRI. This also stresses the need to develop national and international guidelines on this topic, with effective strategies that are based on the best available evidence.

PART II: Medical imaging

There are three indications to request diagnostic imaging for the surgical workup of a patient with diagnosed or suspected FRI.

1) to acquire more certainty about the presence or absence of FRI;
2) to image the surgically relevant details of the disease such as its extension and the presence of sequestra, cloacae, sinus tracts and/or subcortical abscesses;
3) to establish the degree of fracture healing and implant stability.

Radiological signs are considered to be a suggestive sign of FRI according to the AO/EBJIS consensus definition. Nuclear imaging is not yet included in this definition but is expected to be in the near future.

Chapter 3 explains the existing nuclear medicine imaging possibilities for diagnosing FRI and how these modalities are able to answer the diagnostic questions of trauma and orthopaedic surgeons. An overview is also provided of which nuclear imaging technique should be used at which time point during the diagnostic pathway.

Chapter 4 reports on the results of a systematic review of the recent literature (from 2000 to 2016) on imaging techniques for diagnosing FRI. Studies that evaluated the accuracy of magnetic resonance imaging (MRI), three-phase bone scintigraphy (TPBS), white blood cell (WBC) or antigranulocyte antibody (AGA) scintigraphy, fluorodeoxyglucose positron emission tomography (FDG-PET), computed tomography (CT) and plain X-rays in diagnosing FRI were considered for inclusion. The literature search identified 3358 original records. After application of strict predefined inclusion criteria, 10 articles were included. WBC/AGA scintigraphy and FDG-PET exhibit good accuracy for diagnosing FRI (sensitivity range 50-100%, specificity range 40-97% versus 83-100% and 51%-100% respectively). The accuracy of both modalities improved when a hybrid imaging technique (SPECT/CT or FDG-PET/CT) was performed. Sensitivity for FDG-PET/CT ranged between 86 and 94% and specificity between 76 and 100%. For WBC/AGA scintigraphy + SPECT/CT this is 100% and 89-97% respectively. The sensitivity of TPBS was high (ranging from 89 to 100%) but the specificity was low (0 to 10%). TPBS is therefore not suitable for diagnosing FRI. MRI has sensitivity values between 82 and 100% and specificity values between 43% and 60%, so it is less suitable in case confirmation or absence of the disease needs to be established. Sensitivity for CT was 47% and specificity 60%. These values are quite low, therefore CT is not the imaging modality of choice for diagnosing FRI. No studies could be selected on the accuracy of plain X-Rays for diagnosing FRI. It was concluded that, based on the best available evidence of the last 16 years, both WBC (or AGA) scintigraphy combined with SPECT/CT and FDG-PET combined with CT have the best accuracy for diagnosing FRI.

Chapter 5 and Chapter 6 review the diagnostic accuracy of WBC scintigraphy + SPECT/CT and ^{18}F-FDG-PET/CT. To this end, two large cohorts of patients with suspected FRI were retrospectively analysed by reassessing all nuclear imaging scans. A robust and uniform reference standard was applied. The influence of recent surgery was also investigated for both imaging techniques, and the diagnostic performance of standardised uptake values (SUVs) in ^{18}F-FDG-PET/CT for diagnosing FRI was established. WBC scintigraphy showed a high diagnostic accuracy (0.92) for detecting FRIs in the peripheral skeleton.

Duration of the time interval between surgery for the initial injury and WBC did not influence the result, which indicates that WBC scintigraphy is accurate shortly after surgery. ^{18}F-FDG-PET/CT has a lower yet still acceptable diagnostic accuracy (0.83), but should not be performed within one month following surgery as this will decrease its reliability significantly. Finally, it was established that SUV measurements can provide additional diagnostic accuracy when added to qualitative ^{18}F-FDG-PET/CT assessment.

Part III: Serum inflammatory markers

Elevated serum inflammatory markers are also a suggestive criterion for the presence of FRI. The most commonly used serum inflammatory markers in orthopaedic and trauma surgery are C-reactive protein (CRP), leukocyte count (LC) and erythrocyte sedimentation rate (ESR). The difficulty with trauma patients is that elevated inflammatory markers can also be due to non-infectious causes such as systemic inflammatory response and postoperative or posttraumatic tissue damage.

In Chapter 7 the individual diagnostic performance of CRP, LC and ESR are established in a large patient cohort. The diagnostic performance of a combination of these markers and the additional value of including clinical parameters predictive of FRI are also calculated. The diagnostic accuracy of CRP, LC and ESR was 0.52, 0.61 and 0.80 respectively. The area under the receiver operating characteristic (AUROC) curve was 0.64 for CRP, 0.60 for LC and 0.58 for ESR. The AUROC of the combined inflammatory markers was 0.63. Serum inflammatory markers combined with clinical parameters resulted in an AUROC of 0.66 as opposed to 0.62 for clinical parameters alone. The outcome of this retrospective study indicates that the added diagnostic value of CRP, LC and ESR for FRI seems to be limited. FRI can still be present when serum inflammatory markers are within normal range. Clinicians should therefore be cautious when interpreting the results of these tests in patients with suspected FRI.

Chapter 8 is a systematic review of the literature on the diagnostic value of CRP, LC and ESR in patients with FRI. A total of 8280 articles were identified but only six could be included. Sensitivity of CRP ranges were 60.0-100.0% and specificity 34.3-85.7% in all articles. The diagnostic value of CRP from four (n=452) articles could be pooled, showing sensitivity and specificity of 77.0% and 67.9% respectively. For LC sensitivity and specificity 22.9-72.6% and 73.5-85.7% respectively in five articles. Four articles (n=415) reporting on LC were pooled, resulting in 51.7% sensitivity and 67.1% specificity. For ESR, sensitivity and specificity ranges were 37.1-100.0% and 59.0-85.0% respectively in five articles. Three articles on ESR were pooled (n=312), showing 45.1% sensitivity and 79.3% specificity. Four articles analysed the value of combined inflammatory markers, reporting an increased diagnostic accuracy. These results could not be pooled due to

heterogeneity. The results of this review show that the serum inflammatory markers CRP, LC and ESR are insufficiently accurate for diagnosing late FRI, therefore they can only be used as a suggestive sign of this condition.

Part IV: Microbiology

Culturing of surgically obtained deep-tissue samples is one of the most important diagnostic steps in FRI management. The culture of phenotypically indistinguishable pathogens from at least two separate deep-tissue/implant specimens is considered a confirmatory criterion for FRI. In addition, the antibiotic susceptibility of the identified pathogens will guide the choice of antimicrobial treatment.

In Chapter 9 the importance of a structured tissue sampling protocol for diagnosing FRI is demonstrated. Despite stricter criteria for establishing the diagnosis FRI, a structured tissue sampling approach for fracture-related infection led to increased microbiological identification with more certainty of causative pathogens compared to a historic *ad hoc* sampling approach. Simple measures such as an adequate number of deep-tissue samples and use of a dedicated surgical sampling kit can be easily implemented in every hospital. This set of measurements will lead to more trustworthy culture results and consequently a more targeted FRI treatment.

Chapter 10 is a systematic review of validation studies on sonication fluid cultures, molecular techniques and histopathology as diagnostic criteria for FRI. Out of 2624 studies, 10 fulfilled the predefined inclusion criteria. Five studies focused on sonication fluid culture, two on PCR and three on histopathology. There is some evidence that sonication fluid culture may be a useful adjunct to conventional tissue culture, but no strong evidence that it is superior to tissue culture or can replace it. With respect to molecular techniques and histopathology the evidence is even less clear. Overall, studies had variable 'gold standard' criteria for comparison and poorly reported culture methods. It was concluded that it is imperative for lab protocols to become standardised and uniform diagnostic criteria to be implemented so that these techniques can be validated for future diagnosis of FRI.

PART V: Implementation and future perspectives

It is important to increase awareness of a structured and uniform approach towards diagnosing (and treatment) of fracture-related infections. More prospective high-quality and sufficiently powered diagnostic trials should also be conducted.

Chapter 11 is a summary of the recently developed Dutch guideline on diagnosis and treatment of FRI. It is written for all providers of care for patients with FRI and is currently

under review for publication in a widely read Dutch medical journal. Aim of this paper is to bring the need for a structured and multidisciplinary approach towards this condition to the attention of a broader medical audience.

Chapter 12 presents the study protocol of the IFI trial (*The accuracy of diagnostic Imaging techniques in patients with a suspected Fracture-related Infection trial).* This trial will be the first to prospectively compare the three commonly used advanced imaging techniques for diagnosing FRI. Primary endpoints are determining the overall diagnostic performances of WBC/ AGA, FDG-PET and MRI in patients with suspected FRI, and establishing the most accurate imaging strategy for diagnosing this disease. The IFI trial is an example of a prospective clinical trial that is designed to improve the quality of scientific evidence and aid in the development of evidence-based diagnostic pathways for FRI. More similar studies are needed to optimise the quality of care for patients with FRI.

PART VI: General discussion

The overall aim of this thesis is to improve the diagnostic process for FRI. In Chapter 13 the available evidence (based on all chapters included in this thesis) is summarised and recommendations are given on how to diagnose this disease. The diagnostic value of clinical parameters, serum inflammatory markers, imaging modalities, histopathological examination, tissue and sonication fluid sampling, and microbiological and molecular biological techniques is discussed. Suggestions on microbiology laboratory operating procedures for FRI are also provided. Overall, there is still limited scientific evidence regarding diagnostic criteria for FRI. It is strongly recommended to continue collection of prospective data utilising a uniform definition to allow validation and comparison of outcome.

NEDERLANDSE SAMENVATTING (DUTCH SUMMARY)

Het is moeilijk om een ziekte te behandelen die niet correct gediagnostiseerd is. Eén van de uitdagingen in orthopedische traumachirurgie is dat het meest accurate en kosteneffectieve diagnostische traject voor het vaststellen van een fractuur-gerelateerde infectie (*fracture-related infection, FRI*) nog niet is vastgesteld. Dit proefschrift heeft als doel om het diagnostische proces voor FRI te verbeteren door de diagnostische modaliteiten die op dit moment beschikbaar zijn te evalueren en de uitkomst hiervan te implementeren in Nederlandse richtlijnen en toekomstig wetenschappelijk onderzoek.

Deel I: Het probleem

In Hoofdstuk 1 wordt de complicatie FRI geïntroduceerd. Verder wordt de nieuwe AO/EBJIS consensus definitie voor FRI besproken en wordt de opzet van dit proefschrift gepresenteerd.

In Hoofdstuk 2 worden de resultaten van een online inventariserende diagnostische survey gerapporteerd. Er waren 346 respondenten (medisch specialisten en arts-assistenten orthopedie, traumachirurgie, musculoskeletale radiologie en nucleaire geneeskunde), allemaal betrokken bij de zorg voor patiënten met FRI. De conclusie van deze studie was dat er op dit moment geen overeenstemming is wat betreft de optimale diagnostische strategie om posttraumatische osteomyelitis (later FRI genaamd) aan te tonen of uit te sluiten. De aangevraagde medische beeldvorming varieerde sterk en geen van de serum inflammatie markers werd beschouwd als erg specifiek voor het diagnosticeren van FRI. De meeste respondenten beschouwden het CRP wel als de meest nuttige laboratorium test voor het diagnosticeren van FRI. De beschikbaarheid en het op de hoogte zijn van een FRI protocol was matig. De resultaten van deze studie ondersteunen de noodzaak van het uitvoeren van prospectieve klinische studies over de optimale diagnostische strategieën voor FRI. Het onderschrijft ook de noodzaak van het ontwikkelen van nationale en internationale richtlijnen over dit onderwerp, waarin kosteneffectieve strategieën zijn gebaseerd op het best beschikbare wetenschappelijk bewijs.

Deel II: Medische beeldvorming

Er zijn drie indicaties om medische beeldvorming aan te vragen bij de chirurgische analyse van een patiënt met (de verdenking op) een FRI.

1) om meer zekerheid te vergaren over het aan – of afwezig zijn van FRI.
2) om de chirurgisch relevante details af te beelden zoals de uitgebreidheid, de aanwezigheid van sequesters, cloacae, sinustrajecten en/of subcorticale abcessen.
3) om de mate van fractuurheling en implantaat stabiliteit te beoordelen.

Hoofdstuk 3 legt de op dit moment beschikbare nucleaire beeldvormende technieken om FRI te diagnosticeren uit, inclusief de manier waarop deze modaliteiten in staat zijn om de vragen van trauma –en orthopedische chirurgen te beantwoorden. Ook wordt er een overzicht gegeven over welke techniek gebruikt dient te worden op welk moment gedurende het diagnostische traject.

In Hoofdstuk 4 worden de resultaten van een systematische analyse van de recente literatuur (van 2000 tot en met 2016) betreffende medische beeldvorming voor het diagnosticeren van FRI gerapporteerd. Studies die de diagnostische accuratesse van *magnetic resonance imaging* (MRI), drie-fasen botscan (*three phase bone scan*, TPBS), leucocytenscan (*white blood cell scintigraphy*, WBC scintigraphy) of antigranulocytenscan (leukoscan of *antigranulocyte antibody* (AGA)) scintigraphy, *fluorodeoxyglucose positron emission tomography* (FDG-PET), *computed tomography* (CT) en conventionele röntgenfoto (X-ray) evalueerden werden beoordeeld voor eventuele inclusie. Het literatuur onderzoek identificeerde 3.358 originele studies. Nadat van tevoren vastgelegde strikte selectie criteria waren toegepast konden 10 artikelen worden geïncludeerd. WBC/AGA scintigrafie en FDG-PET lieten een goede accuratesse voor het diagnosticeren van FRI zien (sensitiviteit varieerde tussen 50 en 100%, specificiteit tussen 40 en 97% versus 83 - 100% and 51% - 100%, respectievelijk. De accuratesse van beide modaliteiten verbeterde wanneer hybride beeldvormende technieken (SPECT/CT & FDG-PET/CT) werden toegepast. Voor FDG-PET/CT varieerde de sensitiviteit tussen 86 en 94% en de specificiteit tussen 76 and 100%. Voor WBC/AGA-scintigrafie + SPECT/CT was dit 100% en 89 - 97% respectievelijk. De sensitiviteit van TPBS was hoog (tussen 89 en 100%), maar de specificiteit was laag (0-10%). TPBS is daarom niet geschikt om FRI te diagnosticeren. MRI heeft een sensitiviteit tussen 82 en 100% en een specificiteit tussen 43 en 60%. Het is daarom minder geschikt in gevallen waarbij de aan– of afwezigheid moet worden vastgesteld. Voor CT was de sensitiviteit 47% en de specificiteit 60%. Deze waardes zijn laag en daarom is de CT niet de diagnostische beeldvormende techniek van eerste keus voor het diagnosticeren van FRI. Er werden geen studies geselecteerd die de diagnostische accuratesse van conventionele röntgenfoto's voor het diagnosticeren van FRI onderzochten. De conclusie luidde dat, gebaseerd op het beste beschikbare wetenschappelijk bewijs van de laatste 16 jaar, zowel WBC (of AGA) scintigrafie gecombineerd met SPECT/CT en FDG-PET gecombineerd met CT de beste diagnostische accuratesse hebben voor het diagnosticeren van FRI.

In Hoofdstuk 5 en Hoofdstuk 6 worden de diagnostische accuratesse van de WBC scintigrafie+ SPECT/CT en de ^{18}F-FDG-PET/CT beoordeeld. Voor dit doel werden twee grote cohorten met patiënten die werden verdacht van het hebben van een FRI retrospectief onderzocht met herbeoordeling van alle scans. Er werd gebruik gemaakt

van een uniforme en robuuste referentie standaard. Er werd ook onderzocht wat de invloed van recente chirurgie was op de uitkomst van de scans en de diagnostische waarde van *Standardized Uptake Values* (SUVs) bij de ^{18}F-FDG-PET/CT werd bepaald. De WBC scintigrafie liet een hoge accuratesse zien (0,92) voor het diagnosticeren van FRI in het perifere skelet. De duur van het tijdsinterval tussen de laatste chirurgische ingreep en de WBCscintigrafie beïnvloedde de resultaten niet. Dit betekent dat de WBC scintigrafie ook betrouwbaar is kort na chirurgie. ^{18}F-FDG-PET/CT heeft een lagere, maar nog steeds acceptabele diagnostische accuratesse (0,83) voor het diagnosticeren van FRI. De FDG PET dient echter niet verricht te worden binnen 1 maand na chirurgie omdat dit de betrouwbaarheid aanzienlijk doet afnemen. Tot slot werd vastgesteld dat SUV-metingen additionele accuratesse kan bieden wanneer het wordt toegevoegd aan de kwalitatieve ^{18}F-FDG-PET/CT beoordeling.

Deel III: Serum inflammatie markers

Verhoogde serum inflammatie markers zijn ook een suggestief criterium voor de aanwezigheid van FRI. De meest gebruikte serum inflammatie markers in de orthopedie en traumachirurgie zijn het C-reactive protein (CRP), het leucocytengetal (LC) en de bezinkingssnelheid van de erythrocyten (BSE). Het moeilijke bij trauma patiënten is dat een verhoging van de inflammatiemarkers ook veroorzaakt kan worden door niet-infectieuze oorzaken zoals systemische inflammatoire response ziektes en postoperatieve of post-trauma weefselschade.

In Hoofdstuk 7 wordt de individuele diagnostische waarde van CRP, LC en BSE bepaald in een groot patiënten cohort. Ook wordt de diagnostische waarde van een combinatie van deze markers en de toegevoegde waarde van het includeren van klinische parameters die voorspellend zijn voor het hebben van een FRI berekend. De diagnostische accuratesse van CRP, LC en BSE was 0,52, 0,61 en 0,80 respectievelijk. De *area under a receiver operating characteristics* (AUROC) curve was 0.64 voor CRP, 0.60 voor LC en 0.58 voor ESR. De AUROC van de gecombineerde inflammatiemarkers was 0.63. Serum inflammatie markers gecombineerd met klinische parameters resulteerde in een AUROC of 0.66 in vergelijking met 0.62 voor alleen klinische parameters. De uitkomst van deze retrospectieve studie laat zien dat de toegevoegde diagnostische waarde van CRP, LC en BSE gering is. FRI kan nog steeds aanwezig zijn wanneer serum inflammatiemarkers binnen de normale waarde vallen. Daarom dienen clinici de resultaten van deze test met terughoudendheid te interpreteren.

Hoofdstuk 8 is een systematische literatuur analyse naar de diagnostische waarde van CRP, LC en BSE bij patiënten met FRI. In totaal werden 8280 artikelen geïdentificeerd waarvan uiteindelijk slechts zes artikelen konden worden geïncludeerd. De sensitiviteit

van CRP varieerde tussen 60.0 en 100.0% en de specificiteit tussen 34.3 en 85.7% in alle geïncludeerde artikelen. De diagnostische waarde van CRP uit vier artikelen (n=452) konden worden samengevoegd dit leverde een sensitiviteit en specificiteit van 77.0% and 67.9% op, respectievelijk. Voor LC was dit 22.9-72.6% en 73.5-85.7%, respectievelijk, in vijf artikelen. De resultaten van eveneens vier artikelen over LC konden worden samengevoegd (n=415) hetgeen een sensitiviteit van 51.7% en een specificiteit van 67.1% opleverde. Voor BSE varieerde de sensitiviteit en specificiteit tussen 37.1-100.0% en 59.0-85.0%, respectievelijk, in vijf artikelen (n=312). De resultaten van drie artikelen over de diagnostische accuratesse BSE voor FRI konden worden samengevoegd hetgeen een sensitiviteit van 45.1% en een specificiteit van 79.3% liet zien. Vier artikelen analyseerden de waarde van een combinatie van serum inflammatie markers en rapporteerden een toename van de diagnostische accuratesse. De resultaten van deze studies konden niet samen worden gevoegd omdat ze te heterogeen waren. Concluderend laat deze review zien dat de serum inflammatiemarkers CRP, LC and BSE niet geschikt zijn om FRI te diagnosticeren. Daarom kunnen zij alleen gebruikt worden als een suggestief criterium voor de diagnose FRI.

Deel IV: Microbiologie

Het kweken van chirurgisch afgenomen diepe weefsel specimens is één van de belangrijkste diagnostische pijlers van de behandeling van FRI. Het kweken van phenotypisch niet van elkaar te onderscheiden pathogenen in minstens twee diepe weefsel/implantaat specimens wordt beschouwd als een bevestigend criterium voor de diagnose FRI. Daarnaast is de antibiotische gevoeligheid van de geïdentificeerde pathogenen bepalend voor de keuze van het type antibiotica tijdens de verdere behandeling.

In Hoofdstuk 9 wordt het belang van een gestructureerd afname protocol van weefsel specimens voor het diagnosticeren van FRI gedemonstreerd. Ondanks het feit dat striktere criteria werden toegepast voor het stellen van de diagnose FRI, bleek een gestructureerd afname protocol van weefsel specimens voor microbiologisch onderzoek te leiden tot verhoogde microbiologische identificatie met meer zekerheid ten aanzien van de causatieve pathogenen in vergelijking met een historisch 'ad-hoc' afname protocol. Simpele maatregelen zoals een adequaat aantal diepe weefsel specimens en het gebruik van een specifiek voor dit doel samengesteld chirurgisch kweeksetje kan eenvoudig worden geïmplementeerd in ieder ziekenhuis. Deze aanpak leidt tot betrouwbaardere kweekuitslagen en daaruit volgend tot een meer gerichte behandeling van FRI.

Hoofdstuk 10 is een systematische literatuur analyse naar validatie studies betreffende de waarde van sonificatievloeistof kweken, moleculaire technieken en histopathologisch onderzoek als diagnosticum voor FRI. Er werden 2.624 studies geïdentificeerd waarvan er uiteindelijk tien werden geïncludeerd. Vijf studies onderzochten de waarde van sonificatievloeistof kweken, twee studies polymerase chain reaction (PCR) technieken en drie studies histopathologisch onderzoek. Er is beperkt bewijs dat sonificatievloeistof kweken een toegevoegde waarde kunnen hebben als zij gecombineerd worden met conventionele weefselkweken, maar er is geen sterk bewijs dat het superieur is aan weefselkweken of deze kan vervangen. Het wetenschappelijk bewijs ten aanzien van de diagnostische waarde van moleculaire technieken en histopathologisch onderzoek is nog mager. In het algemeen hadden de studies variabele 'gouden standaard' criteria hetgeen het moeilijk maakte om uitkomsten te vergelijken. Ook werden de resultaten van de kweekmethoden matig gerapporteerd. De conclusie van deze studie was dat zowel gestandaardiseerde laboratorium protocollen als uniforme diagnostische criteria geïmplementeerd dienen te worden om de in deze studie onderzochte technieken in de toekomst voor FRI te kunnen valideren.

Deel V: Implementatie en toekomstperspectieven

Het is belangrijk dat het bewustzijn ten aanzien van een gestructureerde en uniforme aanpak van de diagnose (en behandeling) van fractuur-gerelateerde infecties toeneemt. Ook moeten er meer grote, prospectieve en kwalitatief hoogwaardige diagnostische onderzoeken worden uitgevoerd.

Hoofdstuk 11 is een samenvatting van de recent ontwikkelde Nederlandse richtlijn over diagnose en behandeling van FRI. Het is geschreven voor alle zorgverleners van patiënten met een FRI en aan de hand van een klinische casus wordt deze richtlijn besproken. Het doel van dit artikel is om de noodzaak van een gestructureerde en multidisciplinaire benadering van deze aandoening onder de aandacht te brengen van een zo breed mogelijk medisch publiek .

In hoofdstuk 12 wordt het onderzoeksprotocol gepresenteerd van de IFI-studie (*The accuracy of diagnostic Imaging techniques in patients with a suspected Fracture-related Infection*). Deze studie zal het eerste onderzoek zijn dat prospectief de drie algemeen gebruikte geavanceerde beeldvormende technieken voor het diagnosticeren van FRI vergelijkt. Primaire eindpunten zijn 1) het bepalen van de diagnostische accuratesse van WBC / AGA scintigrafie FDG-PET en MRI bij patiënten met verdenking op een FRI en 2) het vaststellen van de meest nauwkeurige beeldvormende strategie voor het diagnosticeren van FRI. De IFI-studie is een voorbeeld van een prospectieve klinische studie die is ontworpen om de kwaliteit van wetenschappelijk bewijs betreffende de

diagnostiek van FRI te verbeteren en om de ontwikkeling van op wetenschappelijk bewijs gebaseerde diagnostische trajecten voor deze aandoening te ondersteunen. Meer vergelijkbare studies zijn nodig om de kwaliteit van de zorg voor patiënten met FRI te optimaliseren.

Deel VI: Algemene discussie

Het doel van dit proefschrift is het verbeteren van het diagnostische proces voor fractuur-gerelateerde infecties.

In Hoofdstuk 13 wordt de beschikbare literatuur (gebaseerd op de hoofdstukken uit dit proefschrift) samengevat en worden aanbevelingen verstrekt ten aanzien van het diagnosticeren van FRI. De diagnostische waarde van klinische parameters, serum inflammatie markers, beeldvormende technieken, histopathologisch onderzoek, weefsel en sonificatie vloeistof kweken, microbiologische en moleculaire technieken wordt besproken. Daarnaast worden suggesties verstrekt betreffende microbiologische laboratorium technieken voor het diagnosticeren van FRI. Samengevat is er nog steeds slechts beperkt bewijs ten aanzien van de diagnostische criteria voor FRI. Het wordt aanbevolen om in de toekomst prospectief gegevens te blijven verzamelen gebruikmakend van een uniforme definitie om validatie en vergelijking van uitkomsten mogelijk te kunnen maken. Tot slot wordt aanbevolen om ook histologisch onderzoek van weefselspecimen en de uitkomsten van nucleaire beeldvorming op te nemen in de FRI consensus definitie.

REVIEW COMMITTEE

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تسألني حبيبتي :

ما الفرق ما بيني وما بين السما ؟

الفرق ما بينكما

أنك إن ضحكت يا حبيبتي

أنسى السماء

Mijn geliefde vraagt mij:

wat is het verschil tussen de Hemel en mij?

Het verschil tussen jullie is,

als jij lacht mijn geliefde,

vergeet ik de hemel.

Nizar Qabbani, Syrische diplomaat en dichter (1923 – 1998)
Vertaling Rim Charfi

DANKWOORD

Geachte lezer,

Dank voor het lezen van dit proefschrift. Of, in ieder geval, dank voor het lezen van dit dankwoord. Dit proefschrift was nooit geschreven als ik niet veel fantastische mensen op mijn pad was tegengekomen die mij allemaal op hun eigen wijze hebben ondersteund. Het was niet alleen een proeve van bekwaamheid maar ook een kwestie van volhouden en geduld hebben. Het is prettig om te promoveren op een onderwerp dat je na aan het hart ligt en het heeft mij veel gebracht. Kennis, inzicht, mooie plannen en vooral mooie momenten met mooie mensen. Ik had nooit kunnen denken dat promoveren zoveel meer is dan alleen kale wetenschap.

Het promotieteam: promotor en copromotoren

Prof. dr. L.P.H. Leenen, beste Loek,
Bedankt voor het vertrouwen en de ondersteuning. En vooral bedankt voor het opzetten van onze prachtige afdeling traumachirurgie waar we blind van elkaar op aankunnen en elkaar steunen. Het geheel is meer dan de som der delen. Ik hoop dat de snor straks krult van plezier. Precies, exact, voor de zekerheid. Je weet maar nooit waar zo'n boekje goed voor is.

Dr. A.W.J.M. Glaudemans, beste Andor,
Ik kan me geen betere begeleider wensen. We kwamen elkaar zes jaar geleden tegen en het klikte. Sindsdien is er geen mail die niet per kerende post beantwoord werd, geen stuk dat ik niet binnen drie dagen gecorrigeerd van je terugkreeg. Niets is onmogelijk en samen is altijd meer. Ik ben je oneindig dankbaar en stiekem wel blij dat je geen chirurg geworden bent!

Dr. F.F.A. IJpma, beste Frank,
Stoïcijns, rustig en betrouwbaar. Als jij zegt dat het goed komt, dan is dat ook zo. Je zit ook vol met goede ideeën om de zorg voor onze patiënten beter te maken. Jij krijgt het voor elkaar om je eigen bekkenplaten te printen en te gebruiken omdat je de commercieel verkrijgbare implantaten niet goed genoeg vindt. Nee is geen optie en je zet door tot je je doel bereikt hebt. Ik ben er trots op dat ik met je mag werken en ik weet dat Utrecht en Groningen samen nog hele mooie dingen gaan doen.

Dr. I.H.F. Reininga, beste Inge,
Jij hoort ook in dit rijtje thuis al vond het UU-promotiereglement helaas van niet. Je was er bij vanaf het eerste uur en altijd bereid om mee te denken. Als geen ander weet je hoe onderzoek werkt en ik ben blij dat je mij geleerd hebt om de eerste stappen op dit pad te zetten.

Hooggeleerde leden van de beoordelingscommissie

Prof. dr. Lam, prof. dr. Castelein, prof. dr. Öner, prof. dr. Verhofstad en dr. Vogely,
Hartelijk dank dat u dit proefschrift heeft willen beoordelen op zijn academische waarde en mijn inspanningen aan uw kritische beoordeling heeft willen onderwerpen.

Staf traumachirurgie van het UMCU

Bewoners van de bezemkast, de OCD-controll room en de jongenskamer. In mijn begintijd in Utrecht dacht ik: wat wordt hier veel gepraat! Maar inmiddels weet ik niet beter. Door de goede sfeer en ons drempelloos bestaan is iedereen altijd op de hoogte van (bijna) alles. Goede communicatie maakt de zorg beter, daar ben ik heilig van overtuigd.

Mirjam de Jong,
Kamergenoot, rots in de branding en nu zelfs promotiemaatje. Wie had dat ooit gedacht. Wat hebben we vaak gelachen en gescholden, op onszelf, op de wereld om ons heen en op dit project. Wees gerust, het boek is af en ééntje is genoeg.

Karlijn van Wessem,
Stille wateren, diepe gronden. Het is goed om te weten dat er op de achtergrond iemand is die alles overziet en met één rake opmerking het geheel in perspectief plaatst.

Falco Hietbrink,
Je bent een oneindige stuiterbal en een geweldige dokter. Gelukkig vertelt Karlijn wanneer je moet werken, Mirjam wat je moet doen en Loek hoe het moet. Bedankt voor je nooit aflatende hulp en goede ideeën, er komen er vast nog veel meer.

Marijn Houwert,
Als jonge hond van de groep duik jij overal op af en in alles zit een stukje. Dank voor je scherpe blik en bijdrage aan dit boekje. Ik heb veel van je geleerd en met name hoe je in de wetenschap doorschakelt naar een hogere versnelling.

Ivar de Bruin,
Jij bent een verademing tussen de OCD-ers. Een frisse wind is goed voor de geest en dankzij jouw inzet heeft de kindertrauma bestaansrecht gekregen. Alle lof hiervoor en ik hoop dat over niet al te lange tijd de vlag in top kan!

Rogier Simmermacher,
Pater Familias. Jouw kritische blik houdt ons scherp en we weten dat je stiekem heel veel verstand hebt van trauma. Je hebt ook als geen ander oog voor de menselijke kant achter de dokter. Dankjewel hiervoor, ik waardeer dit zeer. En die snor stond je goed.

Ingrid Norder en Gioya Bouwman,
Geen staf functioneert zonder goede ondersteuning. Altijd vrolijk, altijd vriendelijk en wat zouden we zijn zonder jullie.

Alle collega's in het UMCU

Verpleging, physician assistants, arts-assistenten, differentianten, fellows, stafleden van alle disciplines en alle andere medewerkers van de afdelingen waar ik dagelijks mee samenwerk en samengewerkt heb. Voor iedere tien namen die ik hier zou noemen zijn er honderd die ik daarmee tekort doe. Jullie werken op de verpleegafdeling, de polikliniek, de SEH, het operatiecomplex en in het calamiteitenhospitaal. En op zoveel andere plaatsen in het UMCU. Ondanks de krapte in de zorg en de soms moeilijk uit te leggen keuzes die gemaakt worden staat de patiënt op de werkvloer altijd voorop. Het is een voorrecht om onderdeel te mogen zijn van dit geweldige team.

Mijn mede-onderzoekers

Janna, Paul en Justin, als ik destijds maar een fractie wist van wat jullie allemaal weten dan had het schrijven van dit proefschrift niet zoveel tijd gekost. Het was geweldig om samen een idee te bedenken, een project op te zetten en dat dan vervolgens te zien transformeren in een manuscript, een presentatie en uiteindelijk een publicatie. Ontzettend knap gedaan, dank jullie wel!

Joost (en ik hoop dat de hooggeleerde heren van de corona niet meelezen): wat weet jij veel van statistiek. Ik heb nog steeds een beetje bijles nodig, heb je een uurtje?

Pien: met Michiel Kaku of Kip Thorne in een donker steegje in Helsinki. Euh, huh?

Monique, mede dankzij jouw enthousiasme is hoofdstuk 6 tot stand gekomen. Dankjewel voor de prettige samenwerking en met hoofdstuk 12 komt er een mooi vervolg!

Staf traumachirurgie van het UMCG

Klaus, dankjewel voor alle steun en mooie momenten tijdens mijn Groningse tijd. Jij was de collega die ik belde als ik een klinisch probleem had. Met veel plezier denk ik terug aan de vele bekkenfracturen die we samen geopereerd hebben en waarbij we niet stopten tot we allebei tevreden waren.

Mariska, ook jij verdient een speciale vermelding. Jij overziet alles en lost problemen op voordat ze er zijn. Dankzij jou blijft het systeem draaien en dan bedoel ik niet alleen de logistiek.

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Mede NVT-bestuursleden

Het is een voorrecht om samen met jullie de Nederlandse Vereniging voor Traumachirurgie te mogen besturen. Wat is het leuk om dit te doen! En Vera, je houdt ons op de juiste wijze, vriendelijke maar beslist, in toom. Zonder jou geen NVT!

My partners in (septic) crime

Mr McNally, dear Martin,
Everyone has an inspirational figure, and you are definitely mine. My AO fellowship at the Oxford Bone Infection Unit was a game-changer for my career – thank you so much. I had never seen anyone so precise and dedicated at handling infections as you, and yet you do it with so much self-deprecating humour that it is always an enjoyable event. The fact that you are a compulsive teacher makes it even better. I hope to be able to spread a little bit of your knowledge in my part of Europe, and join you in your quest to improve the care for patients with FRI.

Dr. Willem-Jan Metsemakers, beste Willem,
Waar was je al die tijd? Het is ongelofelijk om te zien wat jij in zo'n kort tijdsbestek voor elkaar hebt gekregen en hoe jij alle bloedgroepen hebt verenigd. Het FRI-consensus project is nu echt van de grond en er is geen houden meer aan. Petje af voor de gestructureerde manier waarop je de groep leidt en iedereen betrekt, ik kijk uit naar de toekomst.

Charles, Bart, Wies, Emma, Pascal, Rob, Miguel, Edwin en Harrie,
Let's do it! De Utrecht BJIU staat te trappelen om het licht te zien. Zodra de deuren opengaan zit de wachtkamer vol, daar twijfel ik niet aan. Ik hoop dat we in staat zijn om met name de logistieke uitdagingen het hoofd te bieden.

Lieve broers en zussen

En uiteraard ook jullie lieve aanhang en nakomelingen.
Wat is het leuk om in een grote familie op te groeien. Never a dull moment en altijd iemand die voor je klaarstaat. Het maakt het leven zoveel waardevoller.

Lieve ouders

Ik weet niet hoe ik jullie ooit kan bedanken voor alle steun en liefde die ik heb gehad. Daardoor ben ik geworden wie ik ben. Mijn doorzettingsvermogen heb ik van jullie, mijn eigenwijsheid trouwens ook. Ik hoop dat jullie het ons straks toestaan om terug te doen wat jullie voor ons hebben gedaan.

Lieve Rim

Mijn allerliefste lief. Het gaat om de kleine dingen. Samen met jou is alles zoveel mooier en ik hoop dat we daar nog oneindig lang mee door gaan. En ja, je mag mijn nummer …

CURRICULUM VITAE

Geertje Govaert was born in 1972 in the Dutch city of 's-Hertogenbosch and raised in the nearby small village of Dussen. After graduating high school (MAVO degree 1990, Rudolf Steiner School in Breda and VWO degree 1991, Teisterbant College in Zeist) she started medical school at Maastricht University (1992-1999). During her studies she escaped the Netherlands as many times as she could, which resulted in internships in Russia, Zimbabwe, the Dominican Republic and Venezuela. Geertje also spent quite a lot of time at the Student Society 'KoKo', where she became a board member and was in charge of running all hospitality (building and bar) duties in 1995-1996.

After obtaining her medical degree in 1999 she started a residency in tropical medicine at the surgical department of Elisabeth Hospital (now Meander Hospital) in Amersfoort. During that year she realised that surgery was her passion, and instead of continuing her tropical training with the obligatory gynaecology rotation she decided to pursue a surgical path. To that end, in March 2000 she started working at the Academic Medical Center (AMC) in Amsterdam, and was soon-after accepted to their surgical training programme (2001-2006). The first 18 months of her training were spent at the AMC (prof. dr. D.J. Gouma), followed by a six months oncology rotation at the Antoni van Leeuwenhoek Hospital in Amsterdam (prof. dr. B. Kroon). She subsequently moved to Rotterdam to continue her surgical training (2003-2006) at the Albert Schweitzer Hospital in Dordrecht (dr. K.G. Tan and dr. R.J. Oostenbroek). In her fifth residency year, Geertje spent four months at St. Frances Hospital in Katete, Zambia (dr. J. van Bruggen †), practicing all aspects of rural surgery, including gynaecology and urology. In her final year of training Geertje specialised in gastrointestinal surgery.

After obtaining her medical degree as a surgeon, in 2007 Geertje joined the non-governmental organisation 'Doctors without borders' (Médicins Sans Frontières, MSF). With MSF she did two rotations, at the Government Hospital in Kambia, Sierra Leone and at the Emergency Hospital in Dohuk, Iraq. In-between she took on a locum position as general surgeon at the Albert Schweitzer Hospital in Dordrecht.

In 2008 Geertje started her subspecialisation as trauma surgeon by becoming a Trauma Fellow in general surgery at Liverpool Hospital in Sydney, Australia (prof. dr. M. Sugrue and dr. S. D'Amour), followed by a two-year fellowship (continued surgical training, Chirurg in Vervolg Opleiding, CHIVO) at Maastricht University Medical Center (prof. dr. P.R.G. Brink). In 2010 she took the European Trauma Examination (UEMS/EBSQ) in Berlin, becoming a Fellow of the European Board of Surgery. In 2011 she obtained her Dutch qualification as trauma surgeon. Because of a poor Dutch job market for surgeons, she

decided to take on another fellow position in Australia, at the orthopaedic/trauma department of Princess Alexandra Hospital in Brisbane (prof. dr. M. Schütz). By the end of 2011 she was appointed trauma surgeon at University Medical Center Groningen (UMCG). Besides her duties in Groningen as a general trauma surgeon, Geertje developed a passion for pelvic fractures and septic surgery. This is where she started the research that accumulated to become this thesis. The defining moment for her career was probably the four-week AO fellowship that she spent in 2015 at the Oxford Bone Infection Unit with Mr. Martin McNally.

Since 2016 Geertje works as a trauma surgeon at University Medical Center Utrecht. Also in 2016 she became a member of the board of the Dutch Trauma Society (Nederlandse Vereniging voor Traumachirurgie).

Geertje is engaged to Rim Charfi.

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The Oxford Dodo

The dodo (*Raphus cucullatus*) is an extinct flightless bird that was endemic to the island of Mauritius, east of Madagascar in the Indian Ocean. Subfossil remains show the dodo was about 1 metre tall and may have weighed 10.6–17.5 kg in the wild. Though the dodo has historically been considered fat and clumsy, it is now thought to have been well-adapted for its ecosystem. The first recorded mention of the dodo was by Dutch sailors in 1598. In the following years, the bird was hunted by these sailors and the invasive species that they brought with them, and its habitat was being destroyed. The last widely accepted sighting of a dodo was in 1662. Its extinction was not immediately noticed, and some considered it to be a mythical creature. The dodos were a curiosity, and some were brought to Europe by wealthy collectors in the early 17th century. One of these birds was exhibited in John Tradescant's London museum. His collections were later left to Elias Ashmole and so came to Oxford, where now only the mummified head and foot remain in the Museum of Natural History.

The author of this thesis obtained a postcard of a painting of a dodo by George Edwards (1694-1773) at the Oxford University Museum of Natural History during her four-week AO fellowship at the Oxford Bone Infection Unit. This visit, which took place in 2015, was an important moment for her career and this postcard a happy memory.

The back cover image is adapted from the painting by George Edwards. In: *A Natural History of Uncommon Birds, and of Some Other Rare and Undescribed Animals*. London, 1743.