■ TRAUMA

The value of quantitative histology in the diagnosis of fracture-related infection

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Aims
This study aimed to investigate the role of quantitative histological analysis in the diagnosis of fracture-related infection (FRI).

Patients and Methods
The clinical features, microbiology culture results, and histological analysis in 156 surgically treated nonunions were used to stratify the likelihood of associated infection. There were 64 confirmed infected nonunions (one or more confirmatory criteria: pus, sinus, and bacterial growth in two or more samples), 66 aseptic nonunions (no confirmatory criteria), and 26 possibly infected nonunions (pathogen identified from a single specimen and no confirmatory criteria). The histological inflammatory response was assessed by average neutrophil polymorph (NPs) counts per high-power field (HPF) and compared with the established diagnosis.

Results
Assuming a cut-off of over five neutrophils per high-power field to diagnose septic nonunion, there was 80% sensitivity and 100% specificity (accuracy 90%). Using a cut-off of no neutrophils seen in any high-power field to diagnose aseptic nonunion, there was a sensitivity of 85% and a specificity of 98% (accuracy 92%).

Conclusion
Histology can be used in a bimodal fashion as a diagnostic test for FRI. The presence of more than five NPs/HPF had a positive predictive value for infected nonunion of 100%, while the complete absence of any NPs is almost always indicative of an aseptic nonunion (positive predictive value of 98%).

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Fracture-related infection (FRI) and nonunion are among the most challenging complications in orthopaedic trauma surgery, particularly when occurring together. The risk of FRI and nonunion depends on the severity of bone and soft-tissue damage, the anatomical location of the fracture, type of fixation, other injuries, and the host's physiology.[1-3] The infection is almost exclusively acquired exogenously, with soft-tissue injury or surgery allowing ingress of pathogens to grow in biofilms on non-living surfaces such as implants or devitalized bone fragments.[4,5] These localized bacterial colonies are often metabolically quiescent, able to evade the host's immune responses and resist antimicrobial therapy. This makes them difficult to identify in microbiological cultures and hard to eradicate.[1,3,6]

As the presence of infection greatly affects the treatment protocol and outcome of a nonunion, it is crucial to detect or exclude it.[7-9] To do this, standardized interdisciplinary diagnostic and treatment approaches are essential. In contrast to prosthetic joint infection (PJI), there are few standardized diagnostic protocols for infection after trauma, a situation further hampered until recently by the lack of an agreed definition of FRI.[1,3] In order to address this issue, an international expert group was recently convened to develop a consensus definition.[10]

The expert panel defined two levels of certainty: confirmatory, where infection is definitely present, or suggestive (Table I). Infection is confirmed if a sinus tract or wound breakdown is present, there is intraoperative purulence, cultures identify phenotypically indistinguishable pathogens from at least two separate deep-tissue or implant specimens, or microorganisms are identified in deep-tissue specimens on specific staining (e.g. Gram stain).

The most recent definitions of PJI include the presence of a significant acute inflammatory cell

Table I. Definition of fracture-related infection (FRI): the presence of at least one confirmatory criterion defines FRI. The presence of a suggestive criterion requires further investigations in order to look for confirmatory criteria[10]

Confirmatory criteria	Suggestive criteria
Fistula – sinus – wound breakdown	Local and systemic signs of infection
Purulent drainage or presence of pus	Radiological signs
Presence of microorganisms in deep-tissue specimens confirmed by histopathological examination*	Pathogenic organism identified by culture from a single deep-tissue/implant specimen
Phenotypically indistinguishable pathogens identified by culture from at least two separate deep-tissue/implant specimens	Persistent, increased, or new onset wound discharge
	New onset joint effusion
	Elevated serum inflammatory makers

*The presence of microorganisms in tissue is confirmed by using specific histological staining techniques for bacteria or fungi (e.g. Gram stain, Ziehl–Neelsen staining)

infiltrate in periprosthetic tissues on histopathological examination as a diagnostic criterion.[11-14] This is demonstrated by identifying generally more than five neutrophil polymorphs (NPs) per high-power (×400 magnification) field (HPF).[12-14]

In contrast to PJI, histopathology studies in FRI are limited; there is a lack of clear scientific evidence and, more specifically, there is no agreement on a cut-off value for NP infiltration, above which FRI can be reliably diagnosed histologically.[15] Due to the lack of evidence on histological diagnosis, the international expert group was unable to include histology as a criterion for the diagnosis of FRI.[10] Simpson et al[16] analyzed patients with 31 infected and 14 non-infected nonunions and reported a sensitivity of 87% and a specificity of 100% by using a cut-off of more than one NPs/HPF for positive histological analysis.

The aim of this study was to investigate the value of histological analysis in the diagnosis of FRI, using the FRI Consensus Definition[10] as a comparative standard.

Patients and Methods

Inclusion criteria. All skeletally mature patients receiving surgical treatment for nonunion of a long bone fracture in The Oxford Bone Infection Unit were eligible to be included in this study. The inclusion criteria required adequate tissue sample collection from the nonunion site at index surgery, defined as three or more separate microbiology samples and one or more histology samples.

Exclusion criteria were any nonunions following pathological fracture, corrective osteotomy, joint fusion, or previous radiation therapy, as well as any nonunion associated with implantation of foreign materials, such as local antibiotic carriers, polymethylmethacrylate cement spacers, induced-membrane techniques, bone substitutes, or bone grafts.

A total of 150 patients (95 male, 55 women) with 156 nonunions were eligible for analysis and included in this study. Their mean age was 49.1 years (18 to 87).

Definition of nonunion and study cohorts. Nonunion was defined as a fracture that lacks potential to heal without further intervention.[17] Fractures had occurred at a mean of 28.5 months (2 to 536) before definitive surgery.

In this study, the cases were allocated, according to the FRI Consensus Definition,[10] to one of the three nonunion study cohorts depending on clinical presentation and microbiology findings, namely: 1) confirmed infected, 2) possibly infected, and 3) aseptic (Table I).

An aseptic nonunion is defined if none of the confirmed or suggestive criteria of nonunion are present.

Data collection. Data were collected on: demographic characteristics, initial injury pattern and fracture treatment (date of injury, affected bone, whether open or closed, method of fixation), clinical course with wound healing disorders, previous fistulas or previous revision surgery, and antibiotic treatment.

The clinical criteria of the FRI Consensus Definition present at index surgery were noted (Table I).[10] Nonunions were further classified on imaging and intraoperatively, according to the Weber and Čech classification,[18] into viable and non-viable nonunions. Types A (hypertrophic), B (eutrophic), and C (oligotrophic) are generally considered to be viable, because there is vascular bone present on both sides of the nonunion. Type D has non-vascular bone on one or both sides of the nonunion but with no segmental defect. Type E has a separate devitalized piece of sequestered bone, and Type F is associated with bone defects.[18] By definition, all infected cases were classified as Cierny–Mader Anatomic Type IV (diffuse). The patients' physiological status was recorded at index surgery as either Class A (no comorbidities), Class B^L (local compromise in the affected limb, including previous surgery), Class B^S (systemic compromise), or Class B^{LS} (local and systemic compromise).[19]

Sampling and microbiological analysis. At nonunion surgery, at least three (usually five) clean deep-tissue biopsies for microbiology and at least one sample for histology (usually two) were collected from the site of possible infection (fracture site, nonunion, necrotic bone fragments, adjacent deep soft tissues, bed of implant).[20] To avoid cross-contamination, each sample was harvested with a separate clean instrument without touching the patient's skin, and was placed immediately in a sterile transport container.[21] Sampling was performed before extensive debridement and before use of suction or diathermy to minimize risk of contamination. Antibiotics were stopped at least two weeks before surgery. In three cases, antibiotics could not be stopped prior to surgery due to systemic sepsis. Antibiotics were given empirically after sampling. If implants were removed during surgery, these were sent to the microbiological laboratory for sonication. The result from sonication was considered as a single separate sample for microbiological diagnosis.

Table II. Complete study cohort: demographic data, past medical history, and viability of nonunion

Characteristic, n (%)	Total	Confirmed infected nonunion	Aseptic nonunion	Possibly infected nonunion
Patients	150 (*100*)	61 (*40.7*)	63 (*42.0*)	26 (*17.3*)
Cases	156 (*100*)	64 (*41.0*)	66 (*42.3*)	26 (*16.7*)
Demographic data				
Mean age, yrs (SD; range)	49.1 (15.8; 18 to 88)	47.4 (17.2; 19 to 82)	50.2 (14.1; 18 to 85)	50.8 (16.5; 25 to 88)
Male gender	100 (*64.1*)	49 (*76.6*)	35 (*53.0*)	16 (*61.5*)
Affected bone				
Femur	47 (*30.1*)	11 (*17.2*)	26 (*39.4*)	10 (*38.5*)
Tibia	64 (*41.0*)	40 (*62.5*)	17 (*25.8*)	5 (*19.2*)
Fibula	4 (*2.6*)	1 (*1.6*)	2 (*3.0*)	1 (*3.9*)
Humerus	27 (*17.3*)	10 (*15.6*)	12 (*18.2*)	7 (*26.9*)
Forearm (radius and/or ulna)	12 (*7.7*)	1 (*1.6*)	8 (*12.1*)	3 (*11.5*)
Clavicle	2 (*1.3*)	1 (*1.6*)	1 (*1.5*)	0 (*0*)
Open fracture*	53/144 (*36.8*)	31/61 (*50.8*)	14/60 (*23.3*)	8/23 (*34.8*)
Fracture fixation				
None	9 (*6.3*)	4 (*6.7*)	5 (*8.3*)	0 (*0*)
Plate	83 (*57.6*)	38 (*62.3*)	31(*51.7*)	14 (*60.9*)
Nail	43 (*29.9*)	16 (*26.2*)	17 (*28.3*)	10 (*43.5*)
External fixator	9 (*3.3*)	2 (*3.3*)	6 (*9.4*)	1 (*4.4*)
K-wire	1 (*0.7*)	0 (*0*)	1 (*1.7*)	0 (*0*)
Plate and nail	11 (*6.6*)	4 (*6.6*)	6 (*9.4*)	1 (*4.4*)
Previous draining sinus*	66/147 (*44.9*)	43/62 (*69.4*)†	17/61 (*27.9*)†	6/24 (*25.0*)
Previous local signs of infection*‡	98/128 (*76.6*)	59/60 (*98.3*)†	28/51 (*54.9*)†	11/17 (*64.7*)
Host–physiology (Cierny–Mader classification)				
A	5 (*3.2*)	1 (1.6)	3 (4.6)	1 (3.9)
B^L	80 (*51.3*)	31 (48.4)	35 (53.0)	14 (53.9)
B^S	1 (*0.6*)	0 (0)	0 (0)	1 (3.9)
B^{LS}	70 (*44.9*)	32 (50.0)	28 (42.4)	10 (38.5)
Mean time from fracture to non-union surgery, mths (SD; range)	28.5 (52.4; 2 to 536)	26.2 (70.9; 2 to 536)	29.6 (32.9; 4 to 169)	32.2 (25.4; 7 to 94)
Weber and Cech type of nonunion§				
Viable				
A	32 (*20.5*)	4 (*6.3*)	22 (*33.3*)	6 (*23.1*)
B	28 (*18.0*)	9 (*14.1*)	11 (*16.7*)	8 (*30.8*)
C	3 (*1.9*)	2 (*3.1*)	1 (*1.5*)	0 (*0*)
Non-viable				
D	43 (*27.6*)	21 (*32.8*)	16 (*24.2*)	6 (*23.1*)
E	22 (*14.1*)	13 (*20.3*)	8 (*12.1*)	1 (*3.9*)
F	28 (*17.9*)	15 (*23.4*)	8 (*12.1*)	5 (*19.2*)

*Data not available in all cases
†Both previous local signs of infection and a previous sinus occurred significantly more often in the history of infected nonunions compared with aseptic nonunions ($p < 0.001$)
‡Previous prolonged wound discharge or local signs of infection occurring between fracture and nonunion surgery: redness or warmth
§More than half of the aseptic nonunions were viable (52%; n = 34), whereas viable nonunions were significantly less common in confirmed infected nonunions (23%; $p < 0.001$)

Histopathology. Tissue samples were fixed in formalin, processed routinely, and 5 µm paraffin-embedded sections cut. These were stained with haematoxylin and eosin (H&E) and Gram stains. The sections of the sampled tissues from each specimen were examined to find areas of inflammation containing the highest number of neutrophils (NPs). At least ten ×400 magnification HPFs in each of these inflamed areas were examined, and the extent of NP infiltration scored into one of four categories: absent NPs; an average of less than one NP/HPF; an average of one to five NPs/HPF; and an average of more than five NPs/HPF. This categorization of the NP infiltrate was applied to all three study cohorts. The examining pathologist (NAA) was blinded to the clinical and microbiological criteria for each patient.

Statistical analysis. The histological diagnosis was analyzed in confirmed infected nonunions and in aseptic nonunions. The results of the two groups were compared and cut-offs were selected that were best able to diagnose the presence of either infected nonunion or aseptic nonunion. The specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV), and the test accuracy of the histological assessment were calculated.

These results were applied to interpret histological findings in those patients with criteria suggestive of infected nonunions and evaluated in confirming or excluding infection.

Data were collected using a Microsoft Excel spreadsheet (Microsoft, Redmond, Washington) and analyzed using SPSS version 20 (IBM Corp., Armonk, New York). All data were considered to be non-parametric. Associations between categorical variables were made using the chi-squared test. A result was considered to be statistically significant with a p-value < 0.05.

Local institutional review board approval was granted as part of a service evaluation and all patients gave written consent for inclusion.

Table III. Confirmed infected nonunion: histological finding dependent on presence of confirmatory diagnostic criteria as well as disease-causing pathogens

Characteristic, n (%)	Total	Histology diagnosis				
		Not diagnostic of infection				Diagnostic of infection
		0 NPs/HPF	< 1 NP/HPF	1 to 5 NPs/HPF	0 to 5 NPs/HPF	> 5 NPs/HPF
Cases	64 (*100*)	1 (*1.6*)	5 (*7.8*)	7 (*10.9*)	13 (*20.3*)	51 (*79.7*)
Criterion						
Sinus	37 (*57.8*)	0	2	5	7	30
Pus	30 (*46.9*)	0	1	2	3	27
Positive microbiology	59 (*92.2*)	1	4	7	12	47
Sinus alone	1 (*1.6*)	0	0	0	0	1
Pus alone	0 (*0*)	0	0	0	0	0
Positive microbiology alone	22 (*34.3*)	1	3	2	6	16
Sinus + pus	4 (*6.3*)	0	1	0	1	3
Sinus + positive microbiology	11 (*17.2*)	0	1	3	4	7
Pus + positive microbiology	5 (*7.8*)	0	0	0	0	5
All three criteria	21 (*32.8*)	0	0	2	2	19
Pathogens						
S. aureus[*]	12 (*18.8*)	0	1	0	1	11
CoNS	14 (*21.9*)	0	1	4	5	9
P. aeruginosa	4 (*6.3*)	0	0	0	0	4
Coliforms[†]	7 (*10.9*)	0	0	1	1	6
Corynebacterium	3 (*4.7*)	0	0	0	0	3
Bacillus spp.	2 (*3.1*)	0	0	0	0	2
Polymicrobial	20 (*31.3*)	1[‡]	1	2	4	16

*Including one case with MRSA
†Coliforms including *Eschericha coli, Klebsiella* spp., *Enterobacter* spp. (including *Morganella morganii*, *Proteus mirabilis*, and *Serratia marcescens*),and *Citrobacter* spp.
‡Coagulase-negative staphylococci and enterococci
NP, neutrophil polymorphs; HPF, high-power field; CoNS, coagulase-negative staphylococci

Table IV. Possibly infected nonunion: histological findings dependent on previous infection and disease-causing pathogens

Characteristic	Total	Histology diagnosis			
		0 NPs/HPF	< 1 NP/HPF	1 to 5 NPs/HPF	> 5 NPs/HPF
Number of cases, n (%)	26 (*100*)	15 (*57.7*)	4 (*15.4*)	1 (*3.8*)	6 (*23.1*)
Pathogens, n (%)					
CoNS	10 (*38.5*)	5 (*33.3*)	3 (*75.0*)	1 (*100*)	2 (*33.3*)
C. acnes	4 (*15.4*)	3 (*20.0*)	0 (*0*)	0 (*0*)	1 (*16.7*)
Bacillus spp.	5 (*19.2*)	3 (*20.0*)	0 (*0*)	0 (*0*)	2 (*33.3*)
Corynebacterium	1 (*3.9*)	0 (*0*)	0 (*0*)	0 (*0*)	0 (*0*)
Polymicrobial	6 (*23.1*)	4 (*26.7*)	1 (*25.0*)	0 (*0*)	1 (*16.7*)

NPs/HPF, neutrophil polymorphs per high-power field; CoNS, coagulase-negative staphylococci

Results

In total, 156 nonunions in 150 patients were analyzed. According to the FRI Consensus Definition, 64 nonunions were confirmed infected, 26 possibly infected, and 66 aseptic (Table II). Surgical factors are summarized in Table II. Within the confirmed infected nonunion group, 20 (31%) of the infections were caused by a polymicrobial infection, whereas coagulase-negative staphylococci (CoNS) 14 (22%) and *Staphylococcus aureus* 12 (19%) were the most common monomicrobial pathogens (Table III).

In all 26 possibly infected nonunions, only low-virulence pathogens were isolated from one tissue or sonication sample. CoNS (ten, 39%), *Bacillus* spp. (five, 19%) and *Cutibacterium acnes* (four, 15%) were the most commonly detected species. In six (23%) of the possibly infected cases, there were at least two phenotypically different organisms cultured (Table IV). In the cases with complete data, 31 of 61 (51%) confirmed infected cases had sustained an open fracture, in contrast to 14 of 60 (23%) aseptic nonunions ($p = 0.0017$).

More than half of the aseptic nonunions were viable (34, 52%), whereas viable nonunions were significantly less common in the confirmed infected nonunion group (15, 23%; $p < 0.001$).

Quantitative histology results in aseptic and confirmed infected nonunions. In the 64 confirmed infected nonunions, histological analysis revealed an acute inflammatory cell response with more than five NPs/HPF in 51 (80%). In the remaining 13 nonunions (20%), NPs were absent in one case (2%); an average of less than one NPs/HPF was found in five cases (8%), and an average of between one and five NPs/HPF was detected in seven cases (11%) (Tables III and V).

In the 66 aseptic nonunions, NPs were completely absent in 56 cases (85%). Smaller numbers of NPs were detected in ten cases (15%) (seven cases with less than one NP/HPF and three

Table V. Histology in nonunions

		Histology diagnosis	
Diagnostic cut-off: > 5 NPs/ HPF		**Not diagnostic for infection**	**Diagnostic for infection**
		≤ 5 NPs/HPF	> 5 NPs/HPF
All nonunions, n (%)	156 (*100*)	99 (*63.5*)	57 (*36.5*)
Confirmed infected nonunions, n (%)	64 (*100*)	13 (*20.3*)	51 (*79.7*)
Aseptic nonunions, n (%)	66 (*100*)	66 (*100*)	0 (*0*)
Possibly infected nonunions, n (%)	26 (*100*)	20 (*76.9*)	6 (*23.1*)
Diagnostic cut-off: 0 NPs /HPF		**Diagnostic for asepsis**	**Not diagnostic for asepsis**
		Absent (0) NPs/HPF	> 0 NPs/HPF
All nonunions, n (%)	156 (*100*)	72 (*46.2*)	84 (*53.8*)
Confirmed infected nonunions, n (%)	64 (*100*)	1 (*1.6*)	63 (*98.4*)
Aseptic nonunions, n (%)	66 (*100*)	56 (*84.8*)	10 (*15.2*)
Possibly infected nonunions, n (%)	26 (*100*)	15 (*57.7*)	11 (*42.3*)

NPs/HPF, neutrophil polymorphs per high-power field

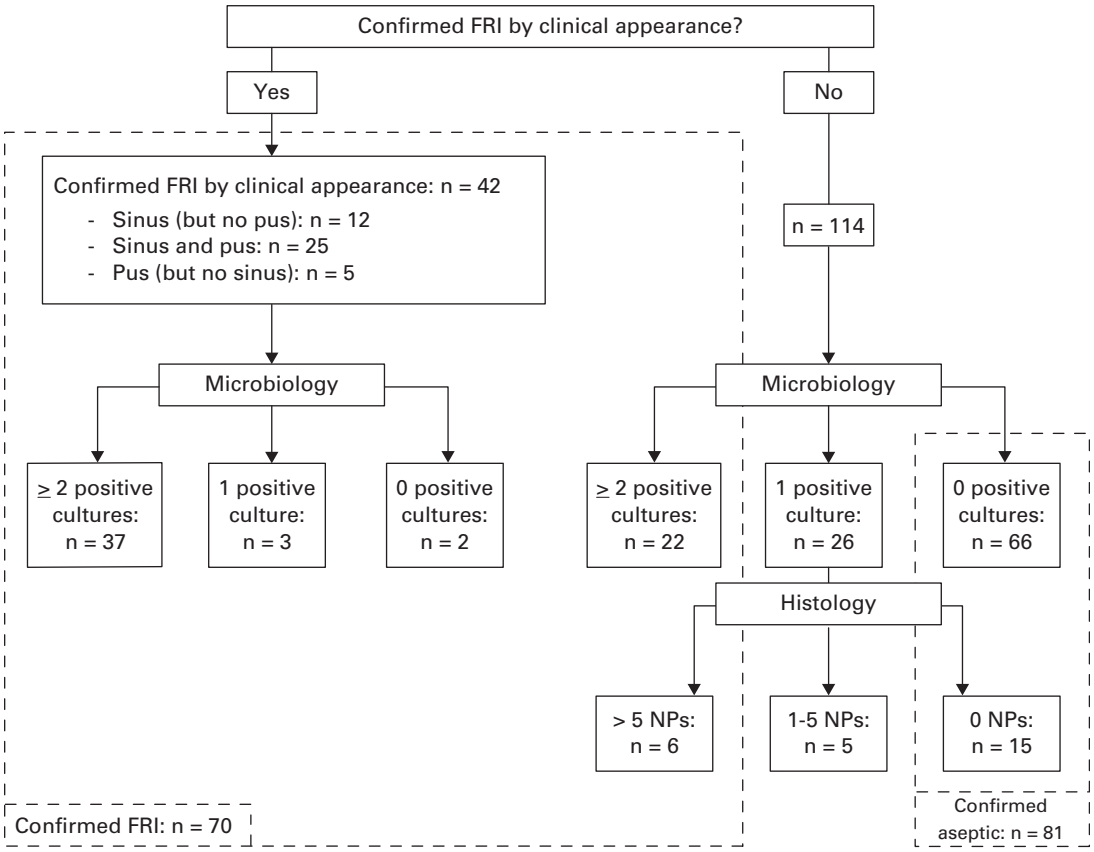

Fig. 1

Diagnostic pathway in nonunions: clinical appearance, microbiology, histology. FRI, Fracture-related infection.

cases with between one and five NPs/HPF). There were no cases of aseptic nonunion with more than five NPs/HPF.

Diagnostic value of quantitative histology. Applying histological analysis with a cut-off of more than five NPs/HPF to diagnose infected nonunion, the 13 'false negative' cases and absence of 'false positive' cases resulted in a sensitivity of 80%, a specificity of 100%, and an overall accuracy of 90%. The PPV for infected nonunion was 100% and the NPV was 84%.

Assuming that to diagnose an aseptic nonunion, neutrophils must be absent across all high-power fields, the one 'false negative' and ten 'false positive' cases would result in a sensitivity of 85%, a specificity of 98%, and an overall accuracy of 92% (Table V). The PPV for aseptic nonunion was 98% and the NPV was 86%.

These results show that histology can be used in a bimodal fashion as a diagnostic test. The presence of more than five NPs/HPF only occurs when infection is present (specificity 100%; PPV 100%) while the complete absence of any NPs is almost always indicative of an aseptic nonunion (specificity 98%; PPV 98%).

Gram staining could detect bacteria in just four nonunions (2.6%). In all of these cases, a marked NP infiltrate (> 5 NPs/

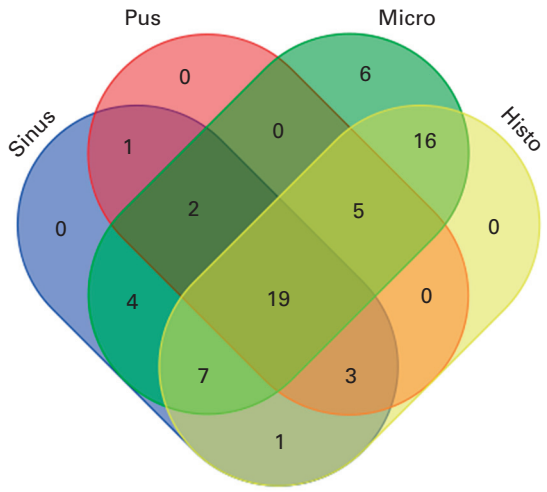

Fig. 2

Presence of confirmatory criteria in 64 infected nonunions: sinus, n = 37; pus, n = 30; microbiology (two or more positive tissue samples), n = 59; histology (more than five neutrophil polymorphs per high-power field), n = 51. Number of cases that: have one criterion, n = 6 (9.4%); share two, n = 22 (34.4%); share three, n = 17 (26.6%); or share four criteria, n = 19 (29.7%).

HPF) was present and microbiology confirmed growth of streptococci or *S. aureus*.

Implementation of histology to aid diagnosis in possibly infected nonunions. Histological analysis revealed more than five NPs/HPF present in six out of the 26 (23%) possibly infected nonunions. In 15 cases (58%), no NPs were detected at all, and in the remaining five (19%) possibly infected nonunions, a few NPs (four cases with less than one NPs/HPF and one case with between one and five NPs/HPF) were seen. If the bimodal NPs cut-offs of no NPs/HPF and more than five NPs/HPF are applied to this possibly infected nonunion group, the histology results can be used to suggest a diagnosis. Consequently, the six cases with more than five NPs/HPF might be regarded as infected (specificity 100%; PPV 100%), whereas the 15 cases with no NPs might be regarded as aseptic nonunions (specificity 98%; PPV 98%). Histology may therefore be able to reduce the number of cases with diagnostic uncertainty from 26 to five cases. This leaves just 5/156 cases (3.2%) where the combination of clinical signs, microbiology, and histology were unable to confidently confirm or exclude infection (Fig. 1).

Discussion

A diagnostic test is only useful if it can be applied to a large majority of cases with high sensitivity and specificity. In using bimodal cut-offs for neutrophil polymorph count rather than a single cut-off, it is possible to achieve greater accuracy than complex imaging tests in FRI[22] (absent NPs to diagnose aseptic nonunion: specificity 98%, PPV 98%; more than five NPs/HPF to diagnose septic nonunion: specificity 100%, PPV 100%). Figure 1 demonstrates that the combination of clinical features, microbiology, and then histological analysis of the cases with uncertain diagnosis (no clinical features and a single microbiological culture) may allow the diagnosis to be established in 151 of 156 nonunions (96.8%). Only five cases remained with an unconfirmed diagnosis. In the 26 cases with uncertain diagnosis, histology identified six unsuspected infections and confirmed aseptic nonunion in 15. This may be a useful adjunct to diagnosis, as these cases often present with difficult to culture organisms (Table IV) or in patients who have been given antibiotics prior to surgery.

Overall, the combination of factors works well to give a composite diagnostic pathway in FRI. In 36 out of 64 confirmed infections (56.3%), three or four diagnostic criteria were positive at index surgery and 89% (57/64) had two or more criteria. (Fig. 2).

This study was conducted to improve the evidence around the diagnostic use of histology in cases of fracture nonunion where there was a suspicion of infection but no definite clinical criteria and culture-negative microbiology. We have focused on established nonunions as earlier, acute FRIs are less difficult to diagnose, often with positive microbiology with virulent organisms or wound breakdown.[3,4,21] In this series, of the 114 cases with no clinical signs of infection, 28 (24.6%) had microbiological and/or histological confirmation of infection. This correlates well with the recent German study that showed that low-grade infection can be present without clinical signs in over 40% of tibial and femoral nonunions.[23]

The results clearly show that high numbers of NPs are strongly associated with infection and a cut-off of more than five NPs/HPF is a reasonable test for use in clinical practice, with a 100% specificity and positive predictive value. However, some definitely infected nonunions (20%) have lower numbers of neutrophils present. This may be due to focal distribution of inflammatory cells,[24] which can be missed when reviewing sample sections. Increasing the number of high-power fields surveyed, or the number of samples harvested at surgery may improve this figure.

Only one case from the confirmed infected nonunion group had no NPs present on histology, which may represent a sampling error at the time of surgery. It is important to provide high-quality samples from all around the nonunion site and implants to reduce the risk of this error. In general, soft tissue from the nonunion, rather than bone specimens, were found to be more representative of the extent of the NPs infiltrate.

A further role is seen when antibiotics have been given before nonunion surgery; while microbiology results may yield false negatives, histology is much more likely to remain positive. In two of our cases where antibiotics were not stopped before surgery due to severe sepsis, this was the case.

We noted also that the presence of NPs in reaming samples was less reliable in confirmed infected nonunions. Reaming produces fairly large volume samples with a mixture of infected and uninfected tissue; while this is unlikely to affect bacterial culture, it may make quantitative histological assessment much more challenging. In this study, there were only nine cases with reaming samples alone, and hence we cannot be conclusive, but we recommend that negative histology of reamings should be interpreted with caution.

This study is limited by the number of cases and the strict inclusion criteria. The results apply only to established nonunion

after fracture and should not be extrapolated to other clinical situations, such as early acute infections. We have also had the benefit of an experienced pathologist (NAA) who specializes in osteoarticular histology; we have, however, tried to keep the pathological assessment as simple as possible by counting only one cell type and not including other more difficult features of bone infection.

In conclusion, we recommend that the presence of an average of more than five NPs per high-power field on histology be considered diagnostic of infection in fracture nonunion and now be added to the FRI Consensus Definition.[10] The complete absence of neutrophils confirms aseptic nonunion in those cases without clinical signs (sinus or pus). The combination of clinical signs, two or more microbiological cultures, and bimodal histological analysis (absent NPs; more than five NPs/HPF) might allow improved diagnostic accuracy in up to 96.8% of cases.

Take home message:

- Diagnosis of infection in fractures can be difficult, particularly in culture-negative cases.
- Use of quantitative histology in a bimodal fashion can improve diagnostic accuracy.
- This combined protocol, with clinical signs, microbiology, and histology, allowed the diagnosis to be confirmed in 97% of cases.

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