Concordance in diabetic foot ulceration: a cross-sectional study of agreement between wound swabbing and tissue sampling in infected ulcers

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Scientific summary

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Background

The identification of pathogens within infected diabetic foot ulcers (DFUs) is necessary to target antibiotic therapy. Wound swabs are commonly used, but most guidelines recommend tissue sampling. There are few large, well-designed, prospective studies, and we lack evidence to advise clinicians on the best technique in DFU management.

We report one 'main study' and three substudies:

- main study: agreement and patterns of disagreement between culture results of swab versus tissue sampling
- substudy 1: independent clinical review of appropriateness of the empirical antimicrobial therapy given to patients, based on swab and tissue cultures
- substudy 2: a pilot comparative study of results of standard plating and culture techniques versus polymerase chain reaction (PCR)
- substudy 3: a follow-up study at 1 year to determine the prognosis for infected DFUs.

Objectives

The primary objective of the COncordance in DIabetic Foot Infection study was to assess agreement (concordance) and patterns of disagreement between culture results from specimens taken by surface swabs and by tissue sampling from patients with a DFU with suspected infection requiring antibiotic therapy.

The secondary objectives were (1) to compare sampling-related adverse events (AEs) and costs; (2) to evaluate whether or not differences in bacterial profiles from specimens obtained by swab versus tissue samples were clinically relevant by asking a panel of clinicians to determine whether or not the reports from each sample would have resulted in a change in clinical management; (3) to assess the concordance between results from specimens with plating and culture techniques and processed by PCR techniques; and (4) to determine the prognosis for infected DFUs by conducting a patient case-note review 12 months after enrolment to determine the clinical outcome of patients with an infected DFU, and to explore prognostic factors related to healing.

Methods

Design

The main study was a multicentre, cross-sectional study involving 400 patients with a DFU and suspected infection. Consenting patients had wound specimens taken by both swab and tissue sampling for plating and culture in order to assess agreement (concordance) and patterns of disagreement.

The secondary objectives were to:

1. Determine the appropriateness of the empirical antibiotic regimen, assessed by a blinded, 'virtual', clinical panel review of the culture results from 247 patients recruited to the main study. Both inter- and intrarater agreement were measured.

2. Compare the results of samples processed by culture methods with those from molecular (PCR) techniques (pilot study on 12 patients).
3. Determine the prognosis and identify risk factors for the healing of infected DFUs, using a 12-month follow-up case-note review on a subsample of 299 patients.

Setting
All patients were enrolled in outpatient DFU clinics or inpatient wards of 25 hospital sites in England.

Patients
Participants were at least 18 years old, with diabetes mellitus and a foot ulcer that the clinician-investigator suspected was infected and planned to treat with antibiotics. Patients were excluded from the study if the clinician deemed it inappropriate to take a tissue or a swab sample or if the patient had previously entered the study.

Procedures
After wound cleansing and debridement, clinicians collected a swab sample from the ulcer using a sterile cotton-tipped swab as per Levine's technique. The clinician then aseptically obtained a tissue sample from the same area of the ulcer bed using sterile equipment (dermal curette or sterile scalpel blade).

Outcome measures
The primary end points for the main study were (1) the reported presence of likely pathogens and an overall summary of pathogens per sample, (2) antimicrobial sensitivities/resistance for likely pathogens and (3) the number of pathogens per specimen. The secondary end points were (1) sampling-related AEs and (2) sampling cost.

The end points in the other substudies were:

- clinical panel review: the appropriateness of the empirical antibiotic therapy
- PCR pilot: the number and identity of pathogens reported by each technique
- prognosis study: (1) the clinical outcomes of patients with an infected DFU and (2) prognostic factors.

Statistical analyses

Agreement study
For pathogens with a prevalence > 8%, overall percentage prevalence, agreement and disagreement, unadjusted kappa, prevalence- and bias-adjusted kappa (PABAK) and McNemar's test for differences are presented. Multinomial regression was used to determine whether or not the overall summary of agreement was influenced by any of baseline factors, and ordinal regression modelling was used to assess the influence of baseline factors on the number of pathogens reported. In both regression analyses, centre was included as a random effect and multiple imputation (MI) was used to impute missing baseline factors.

Clinical panel review substudy
McNemar's test was used to identify if one sample identified significantly more patients requiring a change/initiation in therapy. Multinomial regression analysis was conducted to evaluate the association between baseline factors on agreement for the requirement for a change/initiation of therapy; reviewer was included in each model as a random effect and MI was used to impute missing baseline data.

Polymerase chain reaction substudy
Overall summaries of the pathogens reported using plating/culture and PCR for swab and tissue samples independently, as well as the pathogens reported using PCR techniques by swab and tissue sample are produced.

Prognosis diabetic foot ulcer infection substudy

A competing risk analysis using cumulative incidence functions was conducted to estimate the cumulative incidence of healing at 12 months, adjusted for lower extremity amputation and death. Exploratory analysis was conducted to model the relationship of baseline factors with the cumulative incidence of healing, using the proportional subdistribution hazards model for competing risks data. MI was used to impute the time of healing for patients whose index ulcer was known to have healed but whose date of healing was unknown, and for patients for whom at least one baseline covariate was missing.

Results

Agreement study

A total of 400 patients consented and were recruited, mostly from outpatient clinics (79.8%). Participants had a median age of 63 years (range 26–99 years), a median duration of diabetes of 15 years (range 2 weeks–57 years), and median duration of the index ulcer of 1.8 months (range 3 days–12 years). Before sampling, 60.3% of patients had been treated with an antimicrobial dressing or agent and 46.8% had received systemic antibiotics. Ulcer grades were (as per the Wagner scale), 34% at grade 1, 33.5% at grade 2 and 32.5% at grade 3 or above. In total, 50.5% of ulcers were neuropathic and 49% were ischaemic/neuroischaemic.

There were 395 evaluable patients (i.e. results were available from both swab and tissue culture). At least one pathogen was reported from swabs in 277 (70.1%) patients and from tissue in 340 (86.1%) patients; this difference of 15.9% [95% confidence interval (CI) 11.8% to 20.1%] was statistically significant (p-value < 0.0001, McNemar's test).

The most frequently reported pathogen groups were Gram-positive cocci (70.6%), Gram-negative bacilli (36.7%), Enterobacteriaceae including coliforms (26.6%), obligate anaerobes (23.8%), Gram-positive bacilli (11.1%). The most frequently reported organisms were *Staphylococcus aureus* [excluding meticillin-resistant *S. aureus* (MRSA)] (35.7%), *Streptococcus* (16.7%), *Enterococcus* (14.9%), coagulase-negative staphylococci (CNS) (12.2%), *Corynebacterium* (9.4%), *Pseudomonas* (8.6%) and MRSA (8.1%).

For the majority of pathogens, the reported prevalence was higher from the tissue than from the swab samples (McNemar's p-value < 0.01), with the exception of *S. aureus*, MRSA and *Pseudomonas*. Disagreement between the results of the two specimen types for *S. aureus* and *Pseudomonas* was symmetrical (i.e. pathogen was reported in one sample but not the other equally). The reported prevalence of MRSA was non-significantly higher in the tissue than in the swab sample 1.0% (95% CI –0.2% to 2.8%; p-value $= 0.2188$).

Overall, there was a difference in the pathogens reported from the two sampling techniques for 58% of patients: swabs reported additional pathogens over tissue samples in 8.1% of patients; tissue samples reported additional pathogens over swabs in 36.7% of patients; and tissue samples and swabs reported additional or different pathogens in 13.2% of patients.

The number of reported pathogens ranged from 0 to 4 in the swab sample and 0 to 6 in the tissue sample. The mean number of reported pathogens in the swab and tissue samples was 1 and 1.5, respectively.

In half (49.6%) of patients, the same number of pathogens were reported for the tissue and swab samples; for 41.5% of patients, the tissue sample reported at least one more pathogen than the swab, and for 8.9% of patients the swab sample reported at least one more pathogen than the tissue sample.

There was no evidence of an association regarding agreement of swab and tissue results with ulcer type, Wagner grade, pre-sampling antibiotic therapy or antimicrobial dressing or agent. However, patients whose ulcers had been present for > 56 days had reduced odds of the tissue sampling reporting more pathogens than the swab sample (odds ratio 0.64, 95% CI 0.43 to 0.95; *p*-value = 0.024).

Bleeding of concern was reported in 30 (7.6%) patients and was attributed to swab sampling in 6 (1.5%) patients and to tissue sampling in 27 (6.8%) patients. Different levels of pain after swab and tissue sampling were reported in 42 (10.5%) patients: 5 (1.3%) patients reported worse pain after swabbing compared with tissue sampling, and 37 (9.3%) patients reported worse pain after tissue sampling versus swabbing.

Clinical panel review substudy

Thirteen study clinician-investigators reviewed results from 247 patients. A total of 30 cases were used to measure inter-rater agreement and 30 more to measure intrarater agreement.

There was 73.3% overall agreement on the requirement for a change in (including initiation of) therapy between swab and tissue samples, with a kappa value of 0.45 (95% CI 0.34 to 0.56) representing moderate agreement. The PABAK of 0.47 similarly represents moderate agreement. There was significant evidence that more tissue than swab samples reported a requirement for a change/initiation in therapy [increase of 8.9% (95% CI 2.65% to 15.3%)].

There was no evidence of an association between patient or ulcer characteristics on the agreement between samples on the requirement for a change in therapy.

Polymerase chain reaction substudy

This study included samples from 14 patients from four centres, of which 12 pairs of samples were evaluated by molecular analysis. For six (50%) patients, the PCR technique reported additional pathogens compared with plating and culture for both swab and tissue samples. In four patients (33.3%), the molecular and traditional culture techniques reported the same pathogens, whereas in the remaining two (16.7%) patients, different pathogens were reported using PCR versus traditional culture reports (with or without overlap). There were no samples in which additional pathogens were reported from the traditional culture compared with the PCR results.

Prognosis substudy

We obtained follow-up information on 299 (74.8%) patients, from case notes, at 12 months after baseline sampling. The index ulcer was reported as having healed in 136 (45.5%) patients; in 13 (9.6%) patients, the index ulcer reoccurred before 12 months. A total of 45 (15.1%) patients died within the 12-month follow-up period, 52 (17.4%) patients had a lower-extremity amputation on the same limb on which the index ulcer was found, and 18 (6.0%) patients had revascularisation surgery. The estimated healing rate was 44.5% (95% CI 38.9% to 50.1%) at 12 months.

The median time to healing of those healed was 4.5 months (range 0.5–12.9 months). Of 13 patients whose ulcers were reported to have reoccurred, the median time to reoccurrence was 1.7 months (range 0.3–10.7 months). Median time to death was 5.6 months (range 0.6–11.5 months), and median time to an ipsilateral amputation was 2 months (range 0.0–10.6 months). Median time to revascularisation surgery was 3.0 months (range 0.1–9.5 months). The estimated healing rate was 44.5% (95% CI 38.9% to 50.1%) at 12 months.

Of the 163 (54.5%) patients whose index ulcer had not healed, 93 (57.1%) were known to be alive and without amputation at 12 months, and were censored at the earliest of their case-note review or at the 12-month follow-up. Amputation occurred for 33 (20.2%) patients alive, and seven (4.3%) patients who had died, by 12-month follow-up, resulting in a competing event at patients' date of amputation. A further 30 (18.4%) patients died (without amputation) before 12 months and thus had a competing event at their date of death.

Multivariable regression analysis with competing risk for amputation or death found the following to have a significant direct association with the cumulative incidence of healing as follows (with a HR > 1 indicating a higher likelihood of healing):

- Patients with a perfusion grade ≥ 2 had a lower incidence of healing compared with a grade 1-to-hazard ratio (HR) of 0.37 (95% CI 0.25 to 0.55).
- Patients with older ulcers (≥ 56 days vs. < 56 days) had a lower incidence of healing: HR 0.55 (95% CI 0.39 to 0.77).
- Patients with a single ulcer had an increased incidence of healing compared with those with more than one ulcer on their index foot: HR 1.90 (95% CI 1.18 to 3.06).
- Older patients had an increased incidence of healing with each year of age: HR 1.02 (95% CI 1.01 to 1.04); however, this is suspected to be a spurious relationship.
- Patients whose wound contained CNS at baseline had an increased incidence of healing: HR 1.53 (95% CI 0.98 to 2.40).

Conclusions

Culture of specimens by tissue sampling rather than by swabbing had a higher yield overall. As tissue samples missed some organisms identified by swabbing, the techniques provide somewhat different, potentially complementary, information. The differences in wound microbiology reported from the two types of specimens appeared to be clinically relevant, as the clinical review panel recommended a change in antibiotic therapy more often when presented with tissue results than when presented with swab results (for paired samples from the same wound). As the relationship between microbiology results and the selected antimicrobial therapy, or its effectiveness, is not fully understood, we cannot conclude that the higher yield from tissue sampling would lead to more appropriate therapy or better patient outcomes.

These results may be attributable to the death of the organisms collected during sampling and hence changes to either transport media or specimen collection practices could potentially increase the yield from swabs when processed for culture. For molecular analysis techniques, swabbing appeared to have a higher yield than tissue, potentially owing to the larger sampling area. Furthermore, specimens obtained by swabbing and tissue sampling are likely to be processed and reported differently by many microbiology laboratories. Given that tissue sampling is associated with higher cost and a slightly higher risk of pain and bleeding, the trade-off between sampling techniques needs to be determined by further research to estimate the impact of various sampling regimens on patient outcomes and antibiotic stewardship practices.

The small substudy of PCR techniques found that they identified more organisms than traditional culture, with the difference being greater for swabs than for tissue sampling (based on arbitrary cut-off levels for PCR reporting). Further research is needed to determine the clinical significance of these additional isolates and appropriate cut-off points for clinical practice.

The 1-year prognosis for patients with an infected DFU was poor in this population, and we confirmed the adverse prognostic effect of the presence of ischaemia, higher ulcer grade and longer ulcer duration. Patients with a single foot ulcer are more likely to experience wound healing than those with multiple ulcers. It remains to be determined the extent to which more rapid or complete characterisation of the infected ulcer flora might lead to earlier, more specific and more effective antibiotic therapy.

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