Corneal biopsy with tissue micro-homogenisation for isolation of organisms in bacterial keratitis

JEREMY DIAMOND, JOHN LEEMING, GEOFF COOMBS, JOHN PEARMAN, ANANT SHARMA, CHRIS ILLINGWORTH, GEOFF CRAWFORD, DAVID EASTY

CLINICAL STUDY

Abstract

Purpose **To evaluate a novel two-stage technique to increase yield of bacteria isolated from infected corneal ulcers.**

Methods **A new blade was designed to remove friable material from infected corneal ulcers. The new blade was used in combination with standard tissue micro-homogenisation equipment in a two-stage technique intended to distribute biopsy samples evenly between relevant agar plates. Patients with presumed-bacterial corneal ulcers underwent sequential corneal sampling using the new two-stage technique and a scalpel blade, used without micro-homogenisation (the order of sampling was varied between two groups). Bacterial isolation rates were compared using the chi-squared test.**

Results **Twenty-four patients with presumed-bacterial corneal ulcers were studied. The overall positive bacterial isolation rate was 88%, with identical bacterial isolation rates for the new two-stage technique and the scalpel blade (71%). The new technique isolated bacteria from three ulcers that had initially been 'sterile' when sampled with a scalpel blade. Polymicrobial infections were identified in two ulcers with the new blade where only a single organism had been identified using the scalpel blade (not significantly different).**

Conclusions **The new two-stage technique shows promise for improving bacterial isolation rates from presumed-bacterial corneal ulcers.**

Key words Bacterial keratitis, Corneal scrape, Infection, Laboratory investigation

Bacterial corneal ulcers represent an important cause of visual loss in patients of all ages. They arise as a consequence of contact lens wear, trauma, adnexal disease, topical steroid use, severe debilitation or ocular surface disorders and cause visual loss as a result of corneal scarring, perforation or endophthalmitis.[1-3] About 30 000 bacterial ulcers are treated per annum in the United States.[4]

Presumed bacterial keratitis may be investigated using laboratory techniques including corneal scraping, Gram or other special stains plus culture of relevant materials (e.g. contact lens cases and solutions), allowing culture-guided therapy to target the causative organism.[5,6] While laboratory investigation represents the gold standard for investigation of bacterial keratitis, some clinicians select antimicrobial treatment on an empirical basis.[7] Justification for empirically guided therapy includes the need to commence treatment at presentation and the availability of effective 'broad-spectrum' topical quinolone antibiotics,[8,9] coupled with the cost and inconvenience of laboratory investigation[10] and the high incidence of culture-negative samples obtained with standard scraping techniques.[11] Although empirically guided therapy may suffice in cases of keratitis caused by antibiotic-susceptible bacteria, there is a risk that resistant bacteria may result in unnecessarily poor visual outcome if the microbiological diagnosis is not made.

This investigation was undertaken to evaluate a new technique designed to increase the yield of micro-organisms from corneal scrapes, thereby reducing the incidence of culture-negative sampling.

Material and methods

A new two-stage technique for scraping infected corneal ulcers was employed. The first stage involved a reverse-cutting, angled 'arrow-head' biopsy blade constructed in hardened titanium (Fig. 1) (Duckworth & Kent, Baldock, UK). The blade was designed to be dragged backwards over the full width of an infected corneal ulcer engaging a 'slice' of epithelium, superficial stromal tissue and debris that would then be collected on the protected blade surface. Capillary forces hold the biopsy material upon

J. Diamond
A. Sharma
C. Illingworth
D. Easty
Bristol Eye Hospital
Bristol BS2 2LX, UK

J. Leeming
Public Health Laboratory
Bristol Royal Infirmary
Bristol BS2 8HW, UK

G. Coombs
J. Pearman
Department of Microbiology
Royal Perth Hospital
Perth 6000
Western Australia

G. Crawford
Lions Eye Institute
Nedlands 6009
Western Australia

Mr J.P. Diamond, PhD ✉
Bristol Eye Hospital
Lower Maudlin Street
Bristol BS2 2LX, UK
Tel: +44 (0)1179 284653
Fax: +44 (0)1179 251421
e-mail: j.p.diamond@bris.ac.uk

This research was undertaken with financial support from Alcon (UK) Ltd

Proprietary interest: none

Received: 29 September 1998
Accepted in revised form: 25 January 1999

Fig. 1. *The biopsy blade.*

the flat blade surface allowing repeated passes of the blade over the ulcer with accumulation of the maximum suitable tissue sample. The second stage involved deposition of the biopsy sample into 80 μl sterile phosphate-buffered saline (PBS) in a 0.2 ml micro tissue grinder (Fig. 2) (Wheaton, Millville, NJ). The biopsy sample was then ground using the mortar supplied to produce a suspension of homogenised corneal epithelium, stroma and debris. The resulting 80 μl sample was then distributed in equal parts between culture plates and microscopy slide using a sterile-tipped micro-pipette.

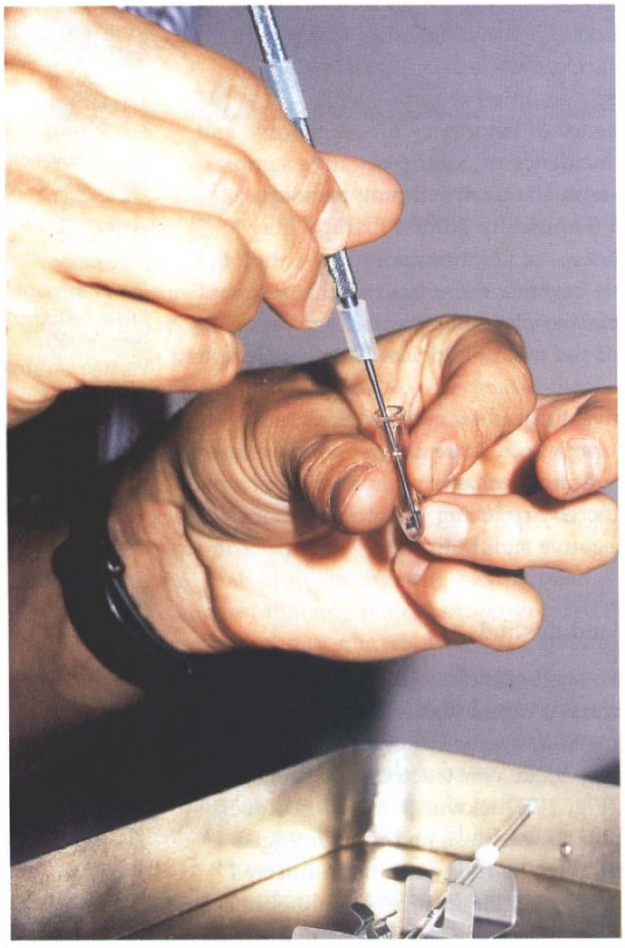

Fig. 2. *The biopsy blade depositing the sample into the Wheaton grinder (Wheaton, Milville, NJ).*

The new technique was tested on patients presenting with previously untreated bacterial corneal ulcers (defined as an area of corneal infiltrate with overlying epithelial defect presumed to be of infectious origin) to the Emergency Departments of the Royal Perth Hospital and Bristol Eye Hospital. Each patient underwent dual sampling with both the new technique and traditional corneal scraping (applied without micro-homogenisation) to enable paired analysis of culture results, thereby improving the likelihood of identifying a variation between the two techniques. Each patient was counselled on the nature of their condition before being asked to give their informed consent for inclusion in the study. Patients were then randomly assigned into two groups (by tossing a coin) before receiving topical anaesthesia with unpreserved oxybuprocaine drops (Minims, Chauvin, Romford, UK). Group A underwent corneal biopsy using the new technique as described above. The 80 μl sample was evenly distributed between blood agar, heated blood agar, Sabouraud's agar and a microscopy slide. Non-nutrient agar seeded with *E. coli* was also utilised if *Acanthamoeba* infection was suspected. Following this initial biopsy each patient underwent a second series of corneal scrapes using a separate no. 11 scalpel blade (Swann-Martin, Sheffield, UK) for each agar plate/microscopy slide. Patients included in group B had these samples taken in reverse sequence, i.e. a full series of scrapes taken with a no. 11 scalpel blade before being biopsied with the new device. Three individuals (J.P.D., A.S., C.I.) were involved in taking samples.

All agar plates and microscope slides were subsequently processed in the standard manner within the microbiology department. The number and type of organisms identified on each plate/slide were collated onto reporting sheets for analysis. Statistical comparisons used the chi-squared test.

Results

A total of 24 patients were included in the study, 12 of whom were male (50%) and 12 female (50%). There were 9 patients in group A and 15 patients in group B, the uneven group sizes reflecting the mode of randomisation. Sixteen patients were recruited in Perth and eight in Bristol.

Table 1 shows bacterial isolation rates for group A while Table 2 gives the data for group B. In group A, micro-organisms were recovered from at least one set of samples in 7 of 9 (78%) patients. All 7 positive samples (100%) were identified using the new technique (which was used first), while 6 (86%) positive samples were also identified by the no. 11 scalpel blade (which was used second) ($p > 0.5$, not significant). One cornea was culture-positive using the new technique but negative when sampled subsequently with the no. 11 scalpel blade. No corneas in group A were culture-negative when sampled

Table 1. *Results for group A patients undergoing corneal scraping with the new device first and with a no. 11 scalpel blade second*

	Micro-organisms recovered using:	
Patient no.	New device	No. 11 blade
A1	Coagulase-negative *Staphylococcus* sp.	–
A2	α-Haemolytic *Streptococcus* sp.	α-Haemolytic *Streptococcus* sp.
A3	*Staphylococcus aureus*	*Staphylococcus aureus*
A4	*Staphylococcus aureus*	*Staphylococcus aureus*
A5	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
A6	Coagulase-negative *Staphylococcus* sp. *Pseudomonas aeruginosa*	Coagulase-negative *Staphylococcus* sp. *Pseudomonas aeruginosa*
A7	Coagulase-negative *Staphylococcus* sp.	Coagulase-negative *Staphylococcus* sp.
A8	–	–
A9	–	–
Total isolates recovered	8	7
Total positive scrapes	7 (78%)	6 (67%)

by the new technique but positive after subsequent sampling with a no. 11 scalpel blade.

In group B, 14 of 15 (93%) patients grew micro-organisms from at least one set of samples. Eleven positive samples (73%) were identified using the no. 11 scalpel blade (which was used first) while 10 (67%) positive samples were identified by the new technique (which was used second) ($p > 0.5$, not significant). Four corneas that were initially positive when sampled using the no. 11 scalpel blade were culture-negative when sampled subsequently with the new technique. In group B there were 3 of 15 (20%) culture-negative samples using the no. 11 scalpel blade that subsequently proved to be positive after a second sample using the new technique. The positive isolation rates for each technique, when used first (i.e. 7/9 and 11/15) were not different ($p > 0.5$, not significant).

One patient in group B (B14) had an amoebic keratitis that was identified using both sampling methods. In the other positive cases the bacteria isolated were typical organisms associated with bacterial keratitis (Tables 1, 2).[12]

When results for 24 patients sampled in groups A and B were combined the new technique produced 17 positive cultures with 25 different organisms (1.5 organism/positive culture) while the no. 11 scalpel blade produced 17 positive cultures with 19 different organisms (1.1 organisms/positive culture), but the difference in recovery rates of polymicrobial cultures by the two sampling methods was not statistically significant ($p > 0.5$).

Discussion

A retrospective audit of 99 corneal scrapes undertaken at Bristol Eye Hospital between 1993 and 1994 demonstrated a positive culture in just 20 samples (20%) (J.P. Diamond, unpublished data 1995). Although a

Table 2. *Results for group B patients undergoing corneal scraping with a no. 11 scalpel blade first and with the new device second*

	Micro-organisms recovered using:	
Patient no.	No. 11 blade	New device
B1	Coagulase-negative *Staphylococcus* sp.	–
B2	*Bacillus* sp.	–
B3	–	Coagulase-negative *Staphylococcus* sp.
B4	Coagulase-negative *Staphylococcus* sp.	–
B5	–	*Flavobacterium* sp.
B6	–	Coagulase-negative *Staphylococcus* sp.
B7	Diphtheroid	Diphtheroid *Staphylococcus* spp. (3 types) *Acinetobacter* sp. α-Haemolytic *Streptococcus* sp.
B8	Diphtheroid	–
B9	Diphtheroid	Coagulase-negative *Staphylococcus* sp. *Bacillus* sp. *Streptococcus sanguis*
B10	*Pseudomonas stutzeri*	*Pseudomonas stutzeri*
B11	*Staphylococous aureus*	*Staphylococcus aureus*
B12	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
B13	–	–
B14	*Acanthamoeba.* sp.	*Acanthamoeba* sp.
B15	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
Total isolates recovered	11	17
Total positive scrapes	11 (73%)	10 (67%)

proportion of those ulcers may have been sterile, a number were presumed to have active microbial infections that could be expected to yield positive cultures in ideal conditions. A review of the literature suggests that such low bacterial isolation rates are not uncommon, with positive culture rates ranging from a low of 37%[13] to a high of 84%.[14]

The recognition that standard corneal scrapes may fail to isolate the organism has prompted other authors to examine new techniques for investigating corneal ulcers, including the use of calcium alginate swabs,[15] confocal microscopy,[16] impression cytology[17] and special stains including immunofluorescent gram staining,[18] calcofluor white[19] and acridine orange.[20] There are a number of reasons why corneal scraping may fail to isolate a micro-organism, including the difficulty sometimes encountered in removing an adequate sample of infected cornea to inoculate an adequate range of different agar plates and microscopy slides. Commonly, a series of ever-smaller biopsies are taken which are distributed to agar plates in sequence, with the last (and smallest) sample usually reserved for microscopy. The majority of the biopsy material may go to an agar plate not best suited for recovery of the pathogen concerned, with inadequate material left for a more appropriate medium. The theoretical advantages of the new technique are threefold: the biopsy blade can accumulate a large volume of infected material upon its surface, homogenisation results in even distribution of this sample between the required number of agar plates and slides, and suspension of the sample in a larger volume would allow the sample to go further where additional media are required (e.g. fungal, viral, *Acanthamoeba* or *Mycobacterium*).

In this study the overall positive bacterial isolation rate was 88%, with similar figures when each technique is reviewed alone (71% for both the new technique and for a no. 11 scalpel blade). These high figures reflect the fact that the ulcers had not previously been treated, that they were selected as likely to be infected and that the scrapes were performed by experienced ophthalmologists rather than residents.

It is not surprising that the second corneal scrapes taken from five cases were culture-negative despite culture-positive primary samples (1 in group A and 4 in group B). This probably reflects the fact that the bulk of friable material available for biopsy was removed with the first sample. More remarkable were the 3 cases (20%) in group B that were negative when sampled with a no. 11 scalpel blade but that proved to be positive when sampled subsequently with the new technique. If this trend were continued the new technique may have the potential to increase the overall bacterial isolation rate in cases of presumed bacterial keratitis. Moreover, two of the three polymicrobial infections were detected only by the new technique (the third was detected by both) and a trend towards increasing the number of different organisms per positive scrape may be clinically significant where those organisms are resistant to current therapy.

The need for corneal scraping is promoted mainly by corneal specialists, who see a selected group of recalcitrant corneal ulcers in tertiary referral centres. Rodman *et al*.[21] reported that the value of corneal scraping is lower in primary referral centres where resistant organisms are less commonly encountered. In a retrospective review of 82 ulcers cultured and treated in the cornea clinic, 8 (10%) required modification of treatment based upon culture data (coupled with failure to respond to empirical therapy), while none of 75 corneal ulcers treated in the general clinic failed to respond to empirical therapy. There is, however, little dispute that severe or recalcitrant corneal ulcers do require full microbiological investigation to allow treatment selection based upon the causative organism. This study has demonstrated a high overall positive bacterial isolation rate where samples are taken by cornea specialists familiar with scrape techniques. The new corneal scrape technique described here shows promise in terms of increasing the isolation rate of micro-organisms and in identifying multiple organisms when present. While this study has not shown a significant increase in yield with the new technique, further work is required to investigate whether the trends demonstrated in this paper are maintained with larger patient numbers and to see whether similar high isolation rates can be achieved by non-corneal specialists using the new apparatus. If so, then this technique could prove valuable in investigating corneal ulcers which are presumed to be infected, particularly where they are severe, sight-threatening or have proved resistant to earlier therapies.

References

1. Parkin B, Turner A, Moore E, Cook S. Bacterial keratitis in the critically ill. Br J Ophthalmol 1997;81:1060–3.
2. Limberg MB. A review of bacterial keratitis and bacterial conjunctivitis. Am J Ophthalmol 1991;112:S2–9.
3. Scott IU, Flynn HW, Feue W, Pfugfelder SC, Alfonso EC, Forster RK, *et al*. Endophthalmitis associated with microbial keratitis. Ophthalmology 1996;103:1864–70.
4. Pepose JS, Wilhelmus KR. Divergent approaches to the management of corneal ulcers. Am J Ophthalmol 1992;114:630–1.
5. Armstrong M. The laboratory investigation of infective keratitis. Br J Biomed Sci 1994;51:65–72.
6. Allan BDS, Dart JKG. Strategies for the management of microbial keratitis. Br J Ophthalmol 1995;79:777–86.
7. McDonnell PJ, Nobe J, Gauderman WJ, *et al*. Community care of corneal ulcers. Am J Ophthalmol 1992;114:531–8.
8. Hyndiuk RA, Eiferman RA, Caldwell DR, Rosenwasser GO, Santos CI, Katz HR, *et al*. Comparison of ciprofloxacin ophthalmic solution 0.3% to fortified tobramycin-cefazolin in treating bacterial corneal ulcers. Ophthalmology 1996;103:1854–63.
9. Ofloxacin Study Group. Ofloxacin monotherapy for the primary treatment of microbial keratitis. Ophthalmology 1997;104:1902–9.
10. McDonnell P. Empirical or culture-guided therapy for microbial keratitis? A plea for data. Arch Ophthalmol 1996;114:84–7.
11. Ficker L, Kirkness C, McCartney A, Seal D. Microbial keratitis: the false negative. Eye 1991;5:549–59.

12. Bower KS, Kowalski RP, Gordon YJ. Fluoroquinolones in the treatment of bacterial keratitis. Am J Ophthalmol 1996;121:712–5.
13. Simcock PR, Butcher JM, Armstrong M, Lloyd IC, Tullo AB. Investigation of microbial keratitis: an audit from 1988–1992. Acta Ophthalmol (Copenh) 1996;74:183–6.
14. McLeod SD, Kolahdouz-Isfahni A, Rostamian K, Flowers CW, Lee PP. The role of smears, cultures and antibiotic sensitivity testing in the management of suspected infectious keratitis. Ophthalmology 1996;103:23–8.
15. Jacob P, Gopinathan U, Sharma S, Rao GN. Calcium alginate swab versus Bard Parker blade in the diagnosis of microbial keratitis. Cornea 1995;14:360–4.
16. Irvine JA, Ariyasu R. Limitations in tandem scanning confocal microscopy as a diagnostic tool for microbial keratitis. Scanning 1994;16:307–11.
17. Yagmur M, Ersoz C, Eroz TR, Varinli S. Brush technique in ocular surface cytology. Diagn Cytopathol 1987;17:88–91.
18. Raychaudhury B, Sharma S, Reddy MK, Das T. Fluorescent gram stain in the microbiologic diagnosis of infectious keratitis and endophthalmitis. Curr Eye Res 1997;16:620–3.
19. Chander J, Chakrabarti A, Sharma A, Saini JS, Panigarhi D. Evaluation of calcofluor staining in the diagnosis of fungal corneal ulcers. Mycoses 1993;36:243–5.
20. Kanungo R, Srinivasan R, Rao RS. Acridine orange staining in early diagnosis of mycotic keratitis. Acta Ophthalmol (Copenh) 1991;69:750–3.
21. Rodman RC, Spisak S, Sugar A, Meyer RF, Soong K, Musch DC. The utility of culturing corneal ulcers in a tertiary referral centre versus a general ophthalmology clinic. Ophthalmology 1997;104:1897–901.