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Acanthamoeba keratitis treatment outcomes compared for drug delivery by protocol versus physician's individualised treatment

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A B S T R A C T

Purpose: To compare Acanthamoeba keratitis (AK) outcomes for treatment delivered using a detailed protocol versus physician's individualised treatment.
Methods: This double cohort study compared the outcomes of these different delivery methods for PHMB 0.02 % and diamidine 0.1 % dual therapy. The primary outcome was the medical cure rate without surgery within 12 months (MCR_12) and the secondary was visual acuity. Any change of treatment, any surgery, or treatment for >12 months was a failure. Outcomes were both unadjusted and adjusted, using multivariable analysis, for baseline differences affecting outcomes. Patients were from two centres in Milan and London treated at different times; the individualised cohort (1991–2012) and per-protocol cohort (2017–2021).
Results: The individualised cohort included 96 and the per-protocol 47 patients. Both unadjusted and adjusted results were similar. The unadjusted outcomes for both centres combined showed significantly improved outcomes for per-protocol treatment with a 1.59-fold improvement in MCR_12 (95 % CI 1.40–1.80, $p < 0.001$) and a 2.1-fold increase in visual acuity ≥20/25 (95 % CI 1.34–3.29, $p < 0.001$). Amongst potential confounding factors examined, neither baseline AK disease stage, treatment centre nor the type of diamidine significantly influenced outcomes.
Conclusions: This study shows significant advantages for the use of protocol delivered versus individualised treatment for AK. The use of evidence-based treatment delivery protocols, like the one used here for AK, might improve outcomes for all causes of microbial keratitis and could offer practitioners and patients the benefit of having an easy-to-follow drug delivery protocol, with known outcomes.

1. Introduction

Protocols for delivery of treatment to keratitis patients are infrequently given in detail except for those in some case series and in randomised controlled treatment trials. In a review of 16 bacterial keratitis treatment trials there was no difference in outcomes and treatment delivery was not considered as a potential bias, probably because treatment protocols were very similar [1]. However, the situation may be quite different for causes of keratitis that are often more difficult to manage like *Acanthamoeba* keratitis (AK) and fungal keratitis (FK), for which treatment times are often weeks or months, and which are often treated with drugs for which the pharmacokinetics have not been established. The lack of a comprehensive evidence based treatment protocol for FK has led to variability in treatment delivery by corneal specialists and difficulty for the non-specialists who often initiate therapy [2]. This issue has been addressed for FK with the publication of the TST (Topical, Systemic, and Targeted Therapy) protocol for FK showing excellent outcomes compared to an historical comparison [2]. Similarly,

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and until now, there has been no comprehensive evidence based treatment protocol for *Acanthamoeba* keratitis (AK). Although a recent treatment guideline has been published it has not been evaluated [3] and several reviews have published brief, but differing, guidelines for drug delivery without supporting evidence [4–6]. No clear treatment termination policy has been described. As a result, corneal specialists use treatment protocols that often differ widely, being individualised for each patient, resulting in treatment frequencies that vary in intensity and length depending on the interpretation of the clinical response, with variable use of adjunctive corticosteroid and using differing treatment termination protocols. This variability makes evaluation of AK treatment outcomes difficult to compare.

Integration of the best available clinical research including the use of evidence-based drug delivery protocols can be expected to lead to care standards, with known outcomes, making treatment delivery easier for both practitioner and patient resulting in higher-quality care for more patients.

Our recent Phase 3 treatment trial [7] used a detailed treatment delivery protocol for AK (https://www.isrctn.com/ISRCTN12199908, Protocol file V2.0 page 16/86) which was evaluated in six centres and for which the ≥85 % cure rate without surgery, or alterations of first line treatment, was the best reported since 2000 and better than those reported in other comparable reports that included more than 100 subjects (see Appendix 1).

The aim of this study was to test the hypothesis that adherence to a detailed treatment delivery protocol will provide better outcomes than practitioners' individualised treatments. This has been evaluated by comparing the outcomes for: (1) AK treatment from a retrospective study [8] in which PHMB 0.02 % plus a diamidine 0.1 % (propamidine or hexamidine) was delivered using practitioners' individualised treatment protocols with (2) those for the protocol treated subjects using PHMB 0.02 % plus propamidine 0.1 % in one arm of a recent randomised controlled trial [7].

2. Methods

The ethical approvals for both the studies from which the individual patient data were derived have been reported [7,8].

2.1. Study design

The study was devised to compare the outcomes for two cohorts of AK patients, each treated with a different drug delivery method using the same drugs, to evaluate our hypothesis that delivery using a detailed protocol, with specifications for drug delivery at each of the critical points encountered in the treatment of the disease to the point of a cure, would provide an improved outcome compared to the variable and individualised treatment delivery that has been usual practice. The study design was planned, as far as possible, to evaluate the effect of these two treatment delivery protocols, rather than any other factors. For these reasons priority was given to minimisation of bias in the design and analysis. Consequently, only centres that used both treatment modalities (Individualised and Protocol) were included in the analysis. This aim was further facilitated by the fact that the protocol treated group results were from a study [7] that had been designed to use the same exclusion criteria, treatments, treatment failure criteria, cure definition, and primary and secondary outcomes as the individualised treatment cohort [8]. To eliminate sources of bias arising from different treatment centres, we ensured that all estimations of the treatment effect were based on within-centre comparisons. Fifteen patients presenting with scleritis and/or hypopyon at baseline in the individualised treatment cohort only were also excluded from the analysis since these complications were identified as powerful prognostic factors in our previous studies and were absent in the Protocol treated cohort. The drugs used were PHMB 0.02 % with a diamidine 0.1 % (either propamidine or hexamidine) dual therapy.

2.2. Data sources

Supplemental Fig. 1 describes the inclusion parameters used to construct the analysis set from the data sources used for this study [7,8]. These were individual patient data from Moorfields Eye Hospital, London and San Raffaele Hospital, Milan having a full record from the onset of symptoms to the end of treatment. The rationale behind the inclusion/exclusion criteria are summarised in Table 1 which summarises the definitions, similarities, differences and outcomes measures for the two study cohorts. The principal differences between the cohorts, apart from the treatment delivery methodology, were: the numbers of subjects, the recruitment period, the clinicians managing the disease, although 11 of the senior staff participated in both studies, most of the total (>44 in the individualised treatment cohort and 44 in the protocol treated) were different, and the diagnostic criteria which included the clinical criteria of keratitis with perineural corneal infiltrates and/or ring infiltrates and/or a clinical course consistent with AK and having a response to anti-amoebic treatment (AAT).

2.2.1 Individualised treatment: There were no detailed treatment delivery protocols in place for managing patients. These administration regimens included differing initiating treatment frequencies and periods, followed by tapering of AAT to as few applications as 2x daily as the clinical signs improved, together with discontinuing AAT either before or after adjunctive topical steroids, when these were used. Table 2 summarises the individualised treatment delivery for a sample (one in every sixth patient of a 48 patient consecutive series) of London patients compared to the protocol specifications for critical treatment points; an expanded version is included in Appendix 2. The complete analysis for each patient is deposited at Mendeley data https://data.mendeley.com/datasets/dwn5829g43/1. The Table shows that every patient had a different AAT and steroid treatment delivery and termination; for example, the initial intensive (hourly day only, or day and night) PHMB dosing frequencies varied from 1 to 38 days with 2 patients having starting doses of 4-6x daily with the same or different frequencies of a diamidine. This was then followed by a frequency reduction for PHMB to 8x daily for most patients from 5 to 86 days, with variable use of diamidines, before a reduction to maintenance frequencies with PHMB to frequencies of 2-6x daily with variable use of diamidines. Steroid use initiation and termination also differed as did the management of recurrences. PHMB was manufactured in Hospital Manufacturing pharmacies (UK) and by SIFI SpA (Italy) to GMP standards.

2.2.2 Protocol treated: a detailed protocol for drug delivery, use of adjunctive therapy and termination parameters, is shown in Fig. 1. Adherence to the protocol was externally monitored by a clinical research organisation throughout the prospective study and although there were minor protocol deviations few were clinically relevant. These included topical steroids started at 15 days as opposed to the protocol mandated earliest start date at 21 days and eye drops being splashed into the eye as opposed to being dropped, before the subject was re-instructed. SIFI SpA produced the PHMB eye drops for both centres.

2.3. Outcome measures

These compared the individualised treatment cohort versus the protocol treated cohort, stratified by centre. The principal outcome measure was the medical cure rate without surgery within 12 months (MCR_12). Secondary outcome measures were best corrected visual acuities, and cure rates for the two different diamidines. The outcomes and reasons for failure are described.

2.4. Statistical analyses

The software package used was Stata software version 17 (StataCorp LP, College Station,

TX). The primary outcome measure was the MCR_12 (proportion)

Table 1
Comparison of the cohorts
Similarities and differences between the "individualised treated" and "protocol treated" cohorts used for the comparison of treatment outcomes from the onset of symptoms to the termination of anti-amoebic treatment.

SIMILARITIES	Individualised treatment versus Protocol treated	
Subjects & treatment centres	At the same eye units in London & Milan[a] None had scleritis or hypopyon at baseline[b]	
Exclusion criteria	Concurrent fungal or herpes simplex virus keratitis	
Anti-amoebic treatment	PHMB 0.02 % with a diamidine 0.1 % dual therapy[c]	
Treatment failure criteria	Change of anti-amoebic therapy (AAT) for any reason, treatment prolonged for ≥12 months, and a need for any type of surgery	
Cure definition	No recurrence of clinical inflammation within 30 days of discontinuation of all anti-amoebic and anti-inflammatory treatment for both cohorts but for which there was an additional check at 90 days for the prospective cohort.	
Primary outcome	Medical cure rate within 12 months (MCR_12)	
Secondary outcomes	Best corrected visual acuity (BCVA), cure rates by *Acanthamoeba* keratitis disease stage[d], treatment centre and diamidine	
DIFFERENCES	**Individualised treatment**	**Protocol treated**
Subject numbers	96 subjects[b]	47 subjects
Treatment period	1992–2012	2017–2021
Number of clinicians	>44 in London and 10 in Milan	44 in London and 10 in Milan
Diagnostic criteria	Histology and/or culture and/or clinical criteria (perineural infiltrates and/or ring infiltrates and/or a clinical course consistent with AK and a response to AAT)	In vivo confocal microscopy, culture and PCR but not clinical criteria alone
Protocol for treatment delivery	**No defined protocol:** individual clinician decision on treatment delivery, use of topical steroids and the clinical criteria for AAT termination	**Protocol driven**: the following critical treatment points are defined in the legend to Fig. 1 & identified by Roman numerals. **I** dosing intensity & period for the initial intensive treatment period & the reduction schedule to the maintenance frequency; **III** protocol for topical steroid use both if used before AK diagnosis & if used later including a the minimum time before their introduction; **IV** clinical resolution criteria, defined as no inflammation & a healed epithelium after 1 month OFF all anti-inflammatory treatment; **V** follow up visit period after clinical resolution; **VI** cure criteria - no relapse after 3 months off treatment; protocols for the management of relapses of infection (**VII**) and exacerbations of inflammation (**VIII**)

[a] From Moorfields Eye Hospital, London and San Raffaele Hospital, Milan.
[b] Of 114 subjects using PHMB plus a diamidine in the individualised treatment study [8] three using PHMB 0.06% & 15 with scleritis or hypopyon at baseline were excluded, leaving 96 subjects in the analysis (see Supp. Fig. 1).
[c] 42/96 (43.8 %) used propamidine 0.1 % and 54/96 (56.3 %) used hexamidine 0.1 % (see Supp. Table 3) for the baseline characteristics for these two subsets.
[d] Stage I corneal epitheliopathy; Stage II corneal epithelial defects, perineural infiltrates or stromal infiltrate; Stage III corneal ring infiltrate with and one or more features of Stage 2 disease.

and was compared between the two study cohorts using a conditional fixed effects Poisson regression model with robust variance, and with the hospital treatment centre specified as a stratification (unbalanced panel) variable. This allowed: (i) ratio estimates i.e. ratio of the two probabilities of cures (Individualised treatment/Protocol treated cohorts) within each stratum (hospital centre); and (ii) an overall probability of cure ratio derived from the 'precision-weighted' average of the stratum-specific result, where the weights are made proportional to inverse of the variance for the cure ratio in each stratum, giving more importance to values that have smaller standard errors. There were two interactions of interest *a priori*: possible modification of the cohort effect (cure ratio of primary interest) by two covariates: AK stage, and treatment centre. These were assessed by comparing the goodness-of-fit (GOF) of Poisson models with and without the interaction term [covariate x study cohort]. Likelihood-ratio tests were used for the comparisons. Covariates selected as candidates for inclusion in the model-building process were all the potential confounders ascertained at baseline, including those that were known prognostic factors for outcome of AK from previous studies: age; AK stage; topical corticosteroids before the start of AAT; and delay in diagnosis, or were regarded as suspected potential confounders (antibiotics and antivirals prior to diagnosis at baseline).

We have not included several factors in this analysis which might be thought to have an effect, but which were not supported by our data or for which we did not have data; baseline visual acuity was not included as both in this, and in our previous studies, it had no predictive effects. Similarly, we have not analysed the microbiology confirmed cases versus in vivo confocal microscopy (IVCM) or clinical diagnosis alone; we have done this for the protocol treated cohort and, as in the randomised trial from which these subjects were derived [7], found no meaningful difference in outcomes (Supplemental Table 1). We do not have the microbiological data to do this analysis for the individualised treatment cohort. Lastly the use of corticosteroids after treatment was started has not been evaluated as this subgroup analysis will be misleading because the effect of steroids might indicate worsening of the AK which would be the root cause of a poor outcome.

A model was first constructed to include all the previously known confounders, with treatment centre having been specified as a stratification variable. The choice of covariates for inclusion in the final model was based on comparing the GOF of the model with or without the suspected confounder using a likelihood ratio test and monitoring the change in the estimated cohort effect. In the absence of a material change in the estimated cohort effect, and no significant change in the GOF, the covariate was excluded from the final model. A similar procedure was used for analysis of the best-corrected final visual acuity outcomes. Fisher's 2-sided exact procedure was used for computation of p-values when comparing the distribution of baseline factors between the two types of study cohort.

3. Results

3.1. Baseline characteristics

Table 3 describes the baseline characteristics of the study participants. These differed principally in 3 of 10 categories: diagnostic delay with a median of 33 days in the individualised treatment cohort vs 22 days in the protocol treated (p 0.012); AK Stage with 2-fold higher proportions having Stage 1 (epitheliopathy) and Stage III (ring abscess) disease in the individualised treatment cohort (p 0.015); and a higher proportion using antibiotics prior to diagnosis in the protocol treated cohort (p 0.005).

Table 2
Protocol delivery specifications compared with treatment delivered by a sample of every 6th case from a consecutive series from the individualised cohort.

Protocol delivery		Individualised treatment delivery (by 10 senior ophthalmologists and their teams) for an 8 case sample summarised by comparison to the protocol delivery specifications. Appendix 2 includes a more detailed summary. Full individual data is available as a spreadsheet at the weblink in the manuscript
Critical treatment points	Specifications	
Initial AAT daily dosing frequency	16x for 5 days	**3 cases used PHMB used 24x daily:** 1 case for 3 days before reduction to 8x daily for 31 days with a diamidine 8x daily**, 1 case** for 1 day reduced to 16x daily for 5 days. **1 case** for 3 days reduced to 16x daily for 5 days **both** with the same frequency of diamidine. **3 cases used 16x daily PHMB for 21, 38 and 14 days** with the diamidine used for 14, 38 and 14 days respectively; **1 case used PHMB 4x daily for 79 days then 16x daily for 5 days** with diamidine used at the same frequency; **1 case used PHMB 6x for 120 days** with diamidine used at the same frequency.
AAT daily dosing frequency reduction schedule	8x for 7 days, 6x for 7 days	**1 case 8x daily for 86 days** and no diamidine; **1 case 8x daily for 30 days reduced to 5x for 21 days** with diamidine at the same frequency; **1 case at 6x daily for 3 days increased to 8x daily for 27 days** before a relapse with no diamidine; **1 case 8x overnight for 8 days then day only for 31** days after which doses varied between 3x and 6x daily for 57 days; **1 case used both drugs 6x daily** throughout, **2 cases used both drugs 8x daily for 5 days**, **1 case** used drugs 6x daily for 14 days
AAT daily dosing frequency for maintenance period	4x thereafter	**5 cases used PHMB 4x daily** of which 1 used the same frequency of diamidine for 98 days, 1 used the PHMB for 63 days with no diamidine, 1 PHMB for 26 days but continued with a diamidine, at variable frequencies, for 231 days, 1 used PHMB for 104 days with no diamidine and 1 used PHMB with no diamidine for 51 days. **1 case used PHMB 2x daily** for 246 days with no diamidine and **1 case used PHMB 3-6x daily** for 161 days **1 case remained on both PHMB and a diamidine 6x daily with both drugs throughout** for 133 days.
Minimum time for steroid drop introduction after start of AAT	Day 20	**2 cases did not use steroid**, **3 cases started steroids at** 14, 29 and 13 days, **1 case started after the start of AAT** but at an uncertain time point, **1 case was concurren**t but probably on steroid at diagnosis, **1 case stopped on diagnosis** and restarted steroid at 41 days after the start of AAT
Steroid drop termination related to AAT termination	30 days before discontinuation of AAT	**1 case** stopped 89 days before AAT discontinued, **2 cases** continued after the AAT discontinued, **1 case** continued steroid after AAT was discontinued but had had a TPK before the diagnosis of AK, **2 cases** stopped steroids at the same time as AAT
Steroid drops when used before the use of AAT	Stop or taper steroids and start an oral (NSAID) drug	**2 cases** were on steroid on diagnosis of AK, **2 cases** continued and **1 case** discontinued
Exacerbations of inflammation	Restart or increase anti-inflammatory treatment WITH 4x daily AAT	None occurred
Protocol for the management of recurrence of AK	Culture for all microbes. Restart with the initial intensive protocol with same AAT if cultures negative for AK or alternative AAT if cultures positive	Two cases only relapsed**. 1 case** initially increased PHMB from 6-8x daily for 27 days then increased to 16x daily for 21 days. **1 case** PHMB 16x and a diamidine 8x daily for 44 days then PHMB reduced to 8x daily for 59 days then 6x daily for 32 days with diamidine 6x daily for 91 days then both PHMB and diamidine 2x daily for another 59 days
Criteria for a cure	Epithelialised with no inflammation after 30 days without AAT and 60 without steroid	All cases cured medically although 3 failed study criteria (cure within 12 months or change of AAT during treatment). **2 cases** steroid not used at all, **1 case** steroid stopped over a month before AAT stopped, **2 cases** had AAT and steroid stopped at the same time, **1 case** had continued steroid after AAT stopped because had a TPK before the diagnosis of AK, **1 case** had steroid stopped 72 days after AAT stopped, **1 case** had steroid continued until having for 337 days without a relapse before an optical PK

3.2. Cure rates

Table 4 shows the difference in proportions cured for both centres combined both unadjusted (raw data) and adjusted for difference in prognostic factors at baseline. Compared to the individualised treatment cohort the protocol treated cohort showed a statistically significant increase in the MCR_12 rates. The overall adjusted improvement in the 12 month cure rate without surgery was 1.59-fold (95 % confidence limits [CI] 1.40–1.80, p-value <0.001) for protocol treatment delivery. The results, stratified for each centre, are provided in Supp. Table 2 and show no substantial differences.

We were concerned that there might be a difference for outcomes between the two diamidines in the individualised treatment cohort, of which propamidine was not available in Italy, whereas both were in use in London. Supplemental Table 3 describes the baseline characteristics for the two diamidines used in the retrospective cohort showing no meaningful differences. Further evidence, showing that the type of diamidine used had no meaningful effect on the outcome of treatment when combined with PHMB 0.02 % is provided in Supplemental Table 4.

Supplemental Table 4a shows that the MCR_12 was almost identical for the two diamidines (cure ratio for propamidine/hexamidine 1.11 (95 % CI 0.78–1.58 p-value 0.568) and Supplemental Table 4b compares the cure proportions between the two hospital centres, where hexamidine was the only diamidine used in Milan, and for which the Milan/London cure ratio was almost identical (unadjusted 0.99 and adjusted 1.06) despite the large difference in the distribution of the two diamidines between the centres.

3.3. Treatment failures

These are described in Supplemental Table 5 together with reasons for failure and outcomes. Criteria for a medical cure were the same in both cohorts although, in the protocol treated cohort, these were more stringent with inclusion of an additional check at 90 days. Forty-two of the 96 (55.3 %) failed in the individualised treatment cohort versus 6/47 (12.8 %) in the protocol treated cohort.

3.4. Best corrected visual acuity

Supplemental Table 6 gives the visual acuity outcomes in 8 categories in Snellen feet from 20/25 to no perception of light; these were generally better for the prospective cohort. Table 5 shows the unadjusted and adjusted analysis, for both centres combined, of those subjects with outcomes resulting in ≥20/25 (normal vision) versus <20/60 (driving level vision in the UK). There was little difference between the overall outcomes for the unadjusted analysis and the analysis adjusted for confounders. The adjusted analysis showed a 2.1-fold (95 % CI 1.34–3.29, p-value <0.001) higher proportion with outcomes ≥20/25 for the prospective cohort. Similarly, for those with BCVA outcomes <20/60 overall the proportions were almost halved in the prospective cohort (outcome ratio 0.51, 95 % CI 0.38–0.68, p-value <0.001). Supp. Table 7 provides the results for each centre; although there were some differences between centres these were in the same direction.

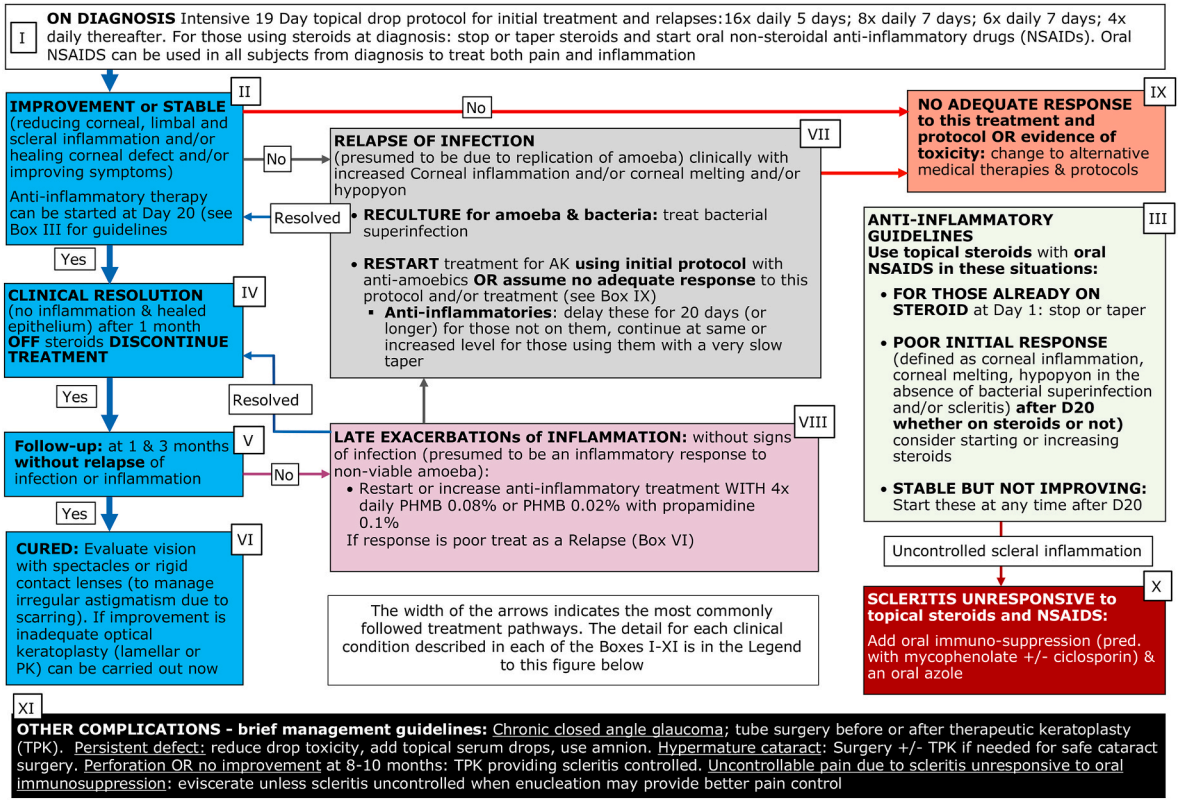
3.5. Other effect modifications (interactions)

Two interactions of *a priori* interest were assessed. There was insufficient evidence for modification of the cohort effect (cure ratio of main interest) by AK stage, or by treatment centre. Likelihood-ratio test p-values were 0.415 and 0.584 respectively.

4. Discussion

The findings of this study are consistent with our hypothesis that the use of a detailed treatment delivery protocol can improve outcomes in AK compared to those resulting from the individualised treatment delivery, currently in widespread use. These results provide data that supports the proposal, in our recently published Phase 3 trial outcome manuscript, that "the trial results may owe as much to the effect of the well-defined drug delivery protocol as to the drugs used" [7]. Some of this improvement may have been due to unidentifiable biases, for which we cannot control, related to the comparison of the prospectively collected dataset for the protocol treated cohort with the retrospectively collected dataset for the individualised treatment cohort. However, having adjusted for all the individual patient variables affecting outcomes available to us we have found substantial potential improvements in clinical outcomes for protocol driven treatment in the proportions cured without surgery increased from 56.3 % to 87.2 % (a 1.59-fold improvement) and BCVA outcomes ≥20/25 improved from 31.3 %–61.7 % (a 2.1-fold improvement).

Our findings have important implications for microbial keratitis therapy which, unlike treatments for many other diseases, are usually managed using broad guidelines for treatment delivery to be varied by the practitioner for each individual patient (individualised treatment) based on their assessment of the clinical response. This is in effect "make it up as you go along" treatment which has been in widespread practice for bacterial keratitis [28], AK and FK [29]. Examples of the resulting wide variability resulting from this type of treatment delivery are provided for the individualised cohort in this study in Table 2 and Appendix 2; it is apparent from this that comparing outcomes for drugs, given the variability in their delivery, is difficult. This type of individualised treatment is practitioner biased and quite distinct from personalised medicine that uses a subjects genetic profile to tailor make treatments [30]. Even for bacterial keratitis, which usually responds to short courses of topical antibiotics, the lack of a defined point for treatment failure, demanding re-evaluation of the diagnosis, is likely to lead to failure to identify, amongst other problems, polymicrobial keratitis (circa 13 % in two studies including several hundred subject culture positive cases [31,32]) for which outcomes are likely to be worse [33]. Polymicrobial infection in AK is quite common with bacterial co-cultures in 14/72 (19.4 %) AK cases [34] and a variety of mixed infections with AK in 26/224 (11.6 %) [35]. Surprisingly, in Cochrane reviews of treatment for fungal and amoebic keratitis, the treatment



(caption on next page)

Fig. 1. This protocol was used in the ODAK (**O**rphan **D**rug for **A**canthamoeba **K**eratitis) Phase III trial comparing PHMB 0.08 % + placebo and PHMB 0.02 % + propamidine 0.1 % for which the details can be found at https://www.isrctn.com/ISRCTN12199908 and in the publication [7] at https://www.ncbi.nlm.nih.gov/pubmed/37802392. It is unchanged here, but the graphic has been simplified and the recommendations for protocol failures and management of complications have been extended for general clinical use. Supplemental Fig. 2 provides this protocol for downloading but including an additional section which expands on the reasoning and evidence base used to develop the protocol.
Clinical conditions are described in a flow chart with boxes labelled with Roman numerals I to XI. The legend below describes the rationale and outcomes from the Trial, and other evidence where available, for each situation. The width of the arrows relates to the frequency of the different clinical situations (see Fig. 1). The **PROTOCOL for treatment delivery** was designed for use with cases that may have had bacterial co-infections but not for those with herpes and fungal co-infections for which we have no evidence from this study.

- **Outcomes using this protocol with PHMB 0.08 % (Akantior) monotherapy:** 56/66 (84.9 %) reported were medically cured (no surgery or change of anti-amoebic treatment [AAT]) within 12 months. One failure in the Trial, due to loss to follow up, was allocated to PHMB 0.08 % and later found to be cured but not included in the Trial statistics because contact could not be made until after the Trial database was locked for analysis giving a medical cure rate of 57/66 (86.4 %). Almost identical results were found for PHMB 0.02 % (the trial formulation of this) with propamidine 1 % (Brolene) of 54/61 (88.5 %).
- **Overall, 111/127 (87.4 %) responded to treatment following the blue pathway (Boxes II, IV, V and VI)**
- **Of the 16 failures in the Trial** (excluding the loss to follow up who was cured on PHMB 0.08 %) changes of treatment were required due to: presumed toxicity 3/127 (2.4 %), inadequate response to trial treatment 10/127 (7.9 %), perforation 2^0 to bacterial keratitis, 1 on treatment at 12 months & 1 concurrent herpes keratitis.

I. ON DIAGNOSIS

In those using topical steroids at trial entry either stop, taper or maintain. See **Box III ANTI-INFLAMMATORY GUIDELINES** and legend for the rationale for adding a oral non-steroidal anti-inflammatory drug (NSAID) when steroid is used.
Treatment is initiated using an intensive 19-day anti-amoebic treatment protocol (in our trial using our PHMB formulations of PHMB 0.02 % + propamidine 0.1 % OR PHMB 0.08 % monotherapy) is given using 1 drop of drug, during the daytime only:
Day 1–5: every hour daytime only hourly drops (16 drops a day) for five days.
Day 6–12: 2 hourly (8 drops a day) for 7 days.
Day 13–19: 3 hourly (6 drops a day) for 7 days.
Day 20: reduce to 4x daily and continue as maintenance therapy thereafter until:
Note: A small proportion of subjects may continue to deteriorate within this period. However, 3 weeks has often been too short a period to decide a whether there is a response; in the Phase 3 trial (127 cases) the response to the treatment was judged inadequate for 10 cases of whom 3 were changed to a different treatment within 2 months (at 27, 33 and 52 days). For the 10 failures the median time to a change of treatment was 107.5 days (range 27–365). For guidelines to alternative treatments and protocols see **IX NO ADEQUATE RESPONSE** to this treatment and protocol.
For most subjects the clinical situation will progress down the left side of the Figure (in the BLUE boxes).

II. IMPROVEMENT or STABLE

Improvement is defined as reducing corneal, limbal and scleral inflammation and/or healing corneal defect and/or improving symptom. These patients continue using AAT 4x daily from Day 20 until **CLINICAL RESOLUTION** (**Box IV**). Those having no deterioration but not improving are STABLE; these subjects may improve over the next few weeks and adjunctive topical steroid may be beneficial (see **Box III**). See Footnote.[1]

III. ANTI-INFLAMMATORY TREATMENT GUIDELINES

Oral NSAIDS can be used in all subjects from diagnosis to treat both pain and inflammation. Oral NSAIDs are effective for scleritis and limbitis as well as for pain relief can be used at any stage as, unlike steroids, there is no evidence that they interfere with the effect of the immune system or AAT in clearing amoebae. If topical steroids are used for corneal/conjunctival inflammation combine these with oral NSAIDs (if not already in use) unless the clinician is confident about the differentiation of scleritis/limbitis from conjunctival inflammation.

a. For those ALREADY ON TOPICAL STEROIDS at Day 1 start oral NSAIDS and stop or taper the steroids.
b. For those with a POOR INITIAL RESPONSE (at D20) defined as corneal inflammation ± corneal melting ± hypopyon in the absence of bacterial superinfection and with or without scleritis then anti-inflammatory therapy may be beneficial, if the cornea is not neurotrophic, on the assumption that most viable *Acanthamoebae* have been killed by D20. For those already on steroids at Day 1 consider increasing these and for those not on steroid consider introducing them with or without oral NSAIDs.
c. FOR PATIENTS WHO ARE STABLE BUT NOT IMPROVING adjunctive anti-inflammatories may be beneficial: start steroids with or without oral NSAIDs at any time after D20.

IV. **CLINICAL RESOLUTION.** For subjects at this point (defined as no inflammation & healed epithelium after 1 month OFF anti-inflammatory treatment) **DISCONTINUE TREATMENT** (topical anti-amoebics and any other anti-microbials). Mild conjunctival inflammation related to drugs or other conditions such as blepharitis are acceptable.

V. **FOLLOW-UP** visits are recommended at 1, 3 and 6 months after clinical resolution. **RELAPSES OF INFECTION** (**Box VII)** are uncommon at this stage (see evidence for this statement in the section on **Termination protocol** below). However, **EXACERBATIONS OF INFLAMMATION (Box VIII)** may occur for several months after treatment termination see **Box VIII** for management.

VI. A **CURE** can be assumed after 3 months with no relapses of inflammation or infection. Patients can have their vision evaluated with spectacles or rigid contact lenses to manage any irregular astigmatism due to scarring. If the vision cannot be adequately corrected, then an optical keratoplasty (lamellar or PK) can be carried out thereafter.

VII. **RELAPSE of infection.** This usually occurs after a period of response to treatment in the maintenance therapy phase. It is identified either by a positive culture, unfortunately very insensitive due to the persistence of deep organisms, supported by an increase in cysts on confocal (also insensitive in severe disease) and most often reliant on clinical criteria alone: development of more severe corneal inflammation, melting, ulceration, hypopyon, development of ring abscess. In the Phase 3 trial of those using PHMB 0.08 %, this developed after 2–3 months in 1 subject, who responded to another intensive course of the same therapy and in 4 subjects, assumed to have a poor response to the treatment or protocol (Trial failures) and whose treatment was changed; see Box **IX** for recommendations

[1] These subjects may improve (meeting the criteria described above) over the next few weeks. Note that in the Phase 3 trial [Reference 7, Fig. S4] images from clinical examples of cured cases showed no change or worse corneal appearances at 30 days both for Stage 1 (11–12) and Stage 2 (21–40) as well as Stage 3 AK (21-04, 21–33, 21–35, 21–36 and 11-09). If there is no improvement but they remain stable, then these patients may be started on anti-inflammatory treatment as described in Box III. However, in the above examples only 11-09 was started on topical steroid and not on this at baseline (when treatment was started); none of the others had topical steroid introduced although 21–40, 21-04, 21–35 and 21–36 were on steroids at baseline and had these withdrawn.

for alternative protocol and treatments. Optimal steroid management for those on steroids is uncertain but we have recommended a very slow withdrawal in one publication [9].

VIII. **LATE EXACERBATIONS OF INFLAMMATION after initial control** occur late, and after treatment termination in a few cases, and are accompanied by mild conjunctival, scleral or corneal inflammation (sometimes coarse anterior stromal infiltrates like those following adenovirus keratitis) occurs late. If symptomatic this responds to topical steroid, but we think MUST be treated with 4x daily anti-amoebic because of anecdotal evidence suggesting **RELAPSE OF INFECTION** in some of these patients probably due to quiescent viable cysts excysting and replicating as trophozoites.

IX. **INADEQUATE RESPONSE TO TREATMENT**: change to alternative medical therapies & protocols. The evidence supporting these is variable but what we think is key data for the following options is cited here:

(1) **Increase the maintenance dose** of PHMB 0.08 % from 4x to 6x or 8x daily - we don't know the optimum therapeutic dose to use after the 19-day intensive treatment period and it may be that higher frequencies are better for some strains of organism, or in some corneas (e.g. damaged oedematous ones). If you suspect a true relapse of infection, but the patient has been doing well to that date, then use another 19-day intensive course (as per protocol) and reduce to a maintenance dose of 6x daily as opposed to 4x daily.

(2) **Add an adjunctive anti-amoebic** (evidence for the value of most of these is well summarized in Kaufman and Tu 2022 [3]) for which there are several choices: propamidine 0.1 % or hexamidine 0.1 % (if propamidine is not available) are effective in combination with a biguanide. Alternatively, chlorhexidine (CHX) 0.02 % for which there is plentiful evidence of effect (see Appendix 1) can be combined with PHMB with probable additive effects [10,11]. Another option is topical voriconazole 1 % which has now been shown to have a clinical effect as monotherapy [12] but which has been widely used for some years as adjunctive therapy [3] with or without systemic voriconazole [3].

(3) **Switch to a new first line anti-amoebic**: CHX 0.2 % (fortified CHX) has been infrequently reported for use in AK [13,14], although used in several cases at Moorfields (unreported) without apparent adverse effect for courses of several weeks; this use followed evidence from its more extensive use in fungal keratitis since 1997 [15]. However, the potential for adverse effects from its prolonged use is currently uncertain; it has caused crystalline deposits, perpetuating persistent defects in 2 cases when combined with either timolol or dexamethasone preservative free [14], and may be antagonistic for *Acanthamoeba* when combined with voriconazole (in vitro) [16] (as opposed to its additive effect in vitro against filamentary fungi [17]). Given the safety of CHX 0.2 % for relatively short-term therapy in fungal keratitis (weeks not months) we think it reasonable for short term use in unresponsive AK providing it is not given with adjunctive timolol or dexamethasone; further evidence is needed before its use for more than a few weeks; intermediate concentrations of CHX (0.04 % and 0.06 %) may prove to be useful in future studies.

(4) **Adjunctive Rose Bengal photodynamic therapy** (RB-PDAT) is a promising treatment with excellent in vitro effects and which has been effective in clinical use for advanced AK resulting in elimination of viable organisms although it may precipitate increased inflammation and corneal perforation in some cases [18]. Currently, and until there is further data from a current clinical trial in *Acanthamoeba* and fungal keratitis (ClinicalTrials.gov ID NCT05110001), it should probably be restricted to use as preparatory treatment for a therapeutic keratoplasty.

(5) **Miltefosine** given systemically is now very costly, having been bought by a US company, resulting in severe restriction of its availability [19]. Its effects from published data (29 eyes in 6 studies [20-25]) have shown 17/29 (59 %) reported as cured (but of whom some also had concurrent RB-PDAT and/or a change of treatment) and 12/29 (41 %) reported as failed. Twenty-two of the 34 (76 %) had an exacerbation of inflammation, 9/29 (31 %) had systemic side effects and 14/29 (48 %) had therapeutic keratoplasties. As a result, its current role in the treatment of AK is uncertain and, despite the FDA granting orphan status to miltefosine for use in AK in the expectation that this would stimulate research, no clinical trials have yet been registered. Topical miltefosine has been used unsuccessfully to date [26].

X. **SCLERITIS UNRESPONSIVE** to treatment in **III.** Add oral immuno-suppression (prednisolone with mycophenolate±ciclosporin) & an oral azole to prevent potential migration of trophozoites into the sclera from the cornea. See reference [27] for recommendations on treatment delivery and outcomes.

XI. **OTHER COMPLICATIONS**: Brief guidelines are for Chronic closed angle glaucoma; tube surgery before or after therapeutic keratoplasty (TPK). Persistent defect: reduce drop toxicity, add topical serum drops, apply amnion. *Hypermature cataract*: surgery±TPK if needed for safe cataract surgery. Perforation OR no improvement at 8–10 months: TPK providing scleritis controlled. Uncontrollable pain due to scleritis unresponsive to oral immuno-suppression: enucleate.

delivery protocol has not been considered as a potential bias when comparing the outcomes of trials [36,37]. This lack of use of defined treatment protocols is unusual in other fields of medicine where evidence based protocols for drug delivery are critical to treatment. Apart from the protocol used in our Phase 3 study [7] the TST protocol for fungal keratitis [2] is the only other evidence-based keratitis protocol for microbial keratitis.

The strengths of our study relate to the similarities between cohorts resulting from the retrospective study (providing the individualised treatment cohort) having been used to plan a the prospective study (providing the protocol treated cohort) at the same centres, with the same exclusion criteria and outcome measures. Despite access to individual patient data for both cohorts, and the use of multivariable analysis to control for differences in both known and potential baseline factors affecting outcomes, the different data collection techniques (prospective versus retrospective) are a limitation and might have resulted in unidentifiable and unmeasurable biases. However, we think our analytical methodology has controlled for the major identifiable biases [38] which are described below.

- Cohort recruitment time periods; the differences could not be controlled for since no periods are shared by both studies. In the individualised treatment cohort, there was a significant trend of higher cure proportions as the years progressed, but the root causes of this trend (including diminishing frequency of Stage 3 disease at baseline, corticosteroids use prior to diagnosis of AK, and delay in diagnosis) were adjusted for in the outcomes analysis as described in the Tables.
- Staff changes; whereas the large numbers of staff involved in treating both cohorts increases the generalizability of the results we think the resulting potential for bias in the assessment of disease severity and outcome assessment has been minimised by having clear cut criteria for both AK disease staging and the outcomes.
- Treatment and management practices; we are not aware of any changes in these, apart from the diagnostic methods over this period. Patients in the individualised treatment cohort included those with a clinical diagnosis as well as those with positive diagnostic test criteria as opposed to those in the protocol treated cohort for whom the inclusion criteria were stricter (IVCM ± culture or PCR); it is difficult to predict what effect this difference might have had on outcomes although we think it unlikely that many had another disease.
- Cure definition; this was the same in both cohorts (no relapse within 30 days of discontinuing anti-amoebic and anti-inflammatory therapy) but was only checked at 90 days in the protocol treated cohort. In the prospective clinical trial, there were no relapses after 30 days in any of the 110 subjects (54 using PHMB 0.02 % plus propamidine 0.1 % and 56 using PHMB 0.08 % monotherapy) meeting the cure definition [7]. A summary of the evidence for the use of the 30 days disease free period of treatment resulting in a cure confirms that relapses after this period are uncommon; this is provided on page 6 of Supp Fig. 2 which includes Fig. 1 but with an additional section

Table 3
Distribution of baseline characteristics for both cohorts. Missing values are ignored in the computations. The data cells contain numbers & (percentages) unless stated otherwise in the row heading.

Baseline Characteristics	Cohort			
	Individualised Treatment n = 96	Protocol Treated n = 47	*Fisher's Exact P*[a]	Totals n = 143
Age				
Mean (sd)	32.0 (12.0)	38.1 (14.8)		34 (13.3)
Median (IQR: Q1, Q3)	29 (21.5, 40.5)	37 (26, 50)	*0.212*[b]	31 (22, 44)
Grouped based on Quartiles of age				
13–22	27 (28.1)	9 (19.1)	*0.246*	36 (25.2)
23–31	26 (27.1)	11 (23.4)		37 (25.9)
32–44	25 (26.0)	11 (23.4)		36 (25.2)
45–75	18 (18.8)	16 (34.0)		34 (23.9)
Gender				
Male	42 (43.8)	18 (38.3)	*0.591*	60 (42.0)
Female	54 (56.3)	29 (61.7)		83 (58.0)
Study period				
Year of diagnosis	1992–2012	2017–2020		
Delayed diagnosis (days)				
Mean (sd)	53.3 (54.9)	34.4 (36.8)		46.9 (50.2)
Median (IQR: Q1, Q3)	33 (16, 77)	22 (9, 39)	***0.012***[b]	29 (13, 61)
Quartiles:				
2–13 days	21 (22.6)	16 (34.0)	*0.088*	37 (26.4)
14–29 days	19 (20.4)	15 (31.9)		34 (24.3)
30–61 days	27 (29.0)	8 (17.0)		35 (25.0)
>61 days	26 (28.0)	8 (17.0)		34 (24.3)
Unknown	*3*	*0*		*3*
AK stage at baseline				
Stage I	21 (21.9)	4 (8.5)	***0.015***	25 (17.5)
Stage II	54 (56.3)	38 (80.9)		92 (64.3)
Stage III	21 (21.9)	5 (10.6)		26 (18.2)
Corticosteroids prior to diagnosis				
No	58 (60.4)	30 (63.8)	*0.718*	88 (61.5)
Yes	38 (39.6)	17 (36.2)		55 (38.5)
Antibiotics prior to diagnosis				
No	34 (35.4)	6 (12.8)	***0.005***	40 (28.0)
Yes	62 (64.6)	41 (87.2)		103 (72.0)
Antivirals prior to diagnosis				
No	59 (61.5)	32 (68.1)	*0.465*	91 (63.6)
Yes	37 (38.5)	15 (31.9)		52 (36.4)
Antifungals prior to diagnosis				
No	95 (99.0)	47 (100)	*> 0.999*	142 (99.3)
Yes (itraconazole)	1 (1.0)	0		1 (0.7)
Study Hospital centre				
London	62 (64.6)	30 (63.8)	*> 0.999*	92 (64.3)
Milan	34 (35.4)	17 (36.2)		51 35.7)

[a] Fisher's exact test compares the frequency distributions.
[b] medians between the two cohorts.

describing the evidence base used to develop the treatment protocol. It is therefore unlikely that there was bias resulting from missed late failures, after 30 days off treatment, in the individualised treatment cohort.

- Baseline criteria; we were only able to control for those for which we had individual patient data for both cohorts; we had not collected social class, race, or smoking behaviour for either cohort, or refraction data for the individualised treatment cohort. However, we don't think it likely that these factors have biased the outcomes.
- Severity of AK; the difference in Stage 3 AK at baseline of 21/96 (21.9 %) of the individualised treatment cohort versus 5/47 (10.6 %) in the protocol treated (Table 3) was controlled for in the cure rate analysis (Table 4).
- Different use of diamidines; this might have been a confounding factor despite their similar effects *in vitro*. [39] However, we think our analysis of the outcomes for the different diamidines in Supplemental Tables 3 and 4 show that this is unlikely to have been a bias.

The numbers in the protocol treated cohort are relatively small limiting the potential for identifying small differences in outcomes but, given that these differences were so large, it is unlikely that all of these were attributable to bias. Whether or not a protocol for drug delivery was used remains one of the substantial differences between the two cohorts.

The beneficial effect of the protocol described here (Fig. 1 and Supp. Fig. 2) extends to the use of PHMB 0.08 % (0.8 mg/ml) reported in the Phase 3 trial [7]. The protocol in Fig. 1 includes the identical treatment delivery protocol to that used in the prospective cohort [7] but adds recommendations for the management of treatment failures and for those developing complications of AK that resulted in their withdrawal from the Phase 3 Trial [7]. These recommendations are evidence based as far as possible.

5. Conclusions

This the only study comparing microbial keratitis outcomes for the use of individualised treatment (delivered variably, adjusting the intensity and length of treatment to the variable clinical response for each individual patient) to that of protocol delivered treatment, in this case for AK. The use of this protocol shows substantial clinical benefits for protocol treated patients compared to those treated with individualised treatment. The medical cure rate improved from 56.3 % to 87.2 % (a 1.59 fold improvement), BCVA ≥20/25 from 31.3 %–61.7 % (a 2.1 fold improvement) and those with BCVA <2/60 reduced from 40.6 % to 19.1 % (halved). The use of a protocol for microbial keratitis treatment delivery may be controversial for many corneal specialists being different from the current practice of individualised treatment even though the latter differs from practice adopted by much of the rest of medicine. Given the benefits to practitioners and patients of having an easy to follow drug delivery protocol, with known outcomes, the potential benefits for the use of this protocol for the treatment of AK should be given some consideration by ophthalmologists. Although we are sure that this protocol could be altered to further improve results, we think it will provide patients and practitioners with a sound evidence base for treatment going forward and hope that it will be adopted by practitioners whose outcomes are not as good as these. We also think that, like

Table 4
Medical cure rate comparison for individualised treatment and protocol treated cohorts
The proportions of *Acanthamoeba* keratitis medical cures within 12 months (MCR_12) in subjects receiving PHMB 0.02 % plus a diamidine for both centres combined comparing individualised treatment to protocol treated. The unadjusted results are shown together with the results of the multivariable analysis which adjusts for differences in baseline prognostic factors. The comparison for each centre independently is included in Supp Table 4; the findings for both were similar.

			Adjusted for confounders [a]			Unadjusted		
Centre	Cohort	MCR_12 (% cured)	% cured Ratio	95 % CI for Ratio	p-value	% cured Ratio	95 % CI for Ratio	p-value
Both centres combined [b]	Individualised treatment	54/96 (56.3)	1 (base)			1 (base)		
	Protocol treated	41/47 (87.2)	1.59	1.40–1.80	<0.001	1.55	1.33–1.81	<0.001

[a] Covariates (evaluated at baseline) adjusted for were age; disease stage (1,2,3); delayed diagnosis days (quartiles of sample); and prior corticosteroids (used or not). Further adjustment for prior antivirals or antibiotics did not alter the estimates of main interest appreciably.
[b] The overall % cured ratio is a weighted average of the stratum (centre)-specific values, where the weights are made proportional to the inverse of the variance for the cure ratio in each stratum, giving more importance to values that have smaller standard errors.

Table 5
Visual acuity outcomes for individualised treatment and protocol treated cohorts at the end of study (after discontinuation of all anti-amoebic therapy) **compared.**
Good visual outcome at the end of study is defined as ≤20/25 best corrected visual acuity (BCVA) or better and poor visual outcome defined as < 20/60 BCVA. Comparisons are for both hospitals combined. Within-hospital for both retrospective and prospective studies by comparison with these overall results are provided in Supp. Table 7.

Visual Acuity Outcomes			Outcome	Outcomes adjusted for confounders [a]			Outcomes unadjusted for confounders		
Centre	Study cohort	N	Final BCVA n (%)	% Ratio	95 % CI for Ratio	p-value	% Ratio	95 % CI for Ratio	p-value
Good visual outcome - Final BCVA ≥20/25									
Both centres combined[b]	Individualised treatment	96	30 (31.3)	1 (base)			1 (base)		
	Protocol treated	47	29 (61.7)	**2.10**	1.34–3.29	0.001	1.98	1.23–3.19	0.005
Poor visual outcome - Final BCVA < 20/60									
Both centres combined[b]	Individualised treatment	96	39 (40.6)	1 (base)			1 (base)		
	Protocol treated	47	9 (19.1)	**0.51**	0.38–0.68	<0.001	0.47	0.36–0.60	<0.001

[a] Covariates (evaluated at baseline) adjusted for were age; disease stage (1,2,3); delayed diagnosis days (quartiles of sample); and prior corticosteroids (used or not). Further adjustment for prior antivirals or antibiotics did not alter the estimates of main interest appreciably.
[b] The overall % cured ratio is a weighted average of the stratum (centre)-specific values, where the weights are made proportional to the inverse of the variance for the cure ratio in each stratum, giving more importance to values that have smaller standard errors.

the TST protocol for FK, the development of similarly well-defined treatment delivery protocols for other microbial keratitis causes, including bacterial keratitis, might improve the outcomes for those diseases. We hope this study results in some debate about the use of protocols for microbial keratitis treatment.

CRediT authorship contribution statement

John K.G. Dart: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Vincenzo Papa:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Data curation. **Paolo Rama:** Writing – review & editing, Investigation, Data curation. **Karl Anders Knutsson:** Writing – review & editing, Investigation. **Saj Ahmad:** Writing – review & editing, Investigation. **Scott Hau:** Writing – review & editing, Investigation. **Sara Sanchez:** Writing – review & editing, Investigation, Data curation. **Antonella Franch:** Writing – review & editing, Investigation, Data curation. **Federica Birattari:** Writing – review & editing, Investigation. **Pia Leon:** Writing – review & editing, Investigation. **Adriano Fasolo:** Writing – review & editing, Investigation, Data curation. **Ewa Mrukwa-Kominek:** Writing – review & editing, Investigation, Data curation. **Katarzyna Jadczyk-Sorek:** Writing – review & editing, Investigation, Data curation. **Fiona Carley:** Writing – review & editing, Investigation, Data curation. **Hossain Parwez:** Writing – review & editing, Investigation, Data curation. **Darwin C. Minassian:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

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Conflict of interest

Vincenzo Papa is an employee of SIFI S.p.A. who sponsored the pharmaceutical development of polihexanide as a licenced therapy for the treatment of *Acanthamoeba* keratitis. The remaining authors have no proprietary or commercial interest in any materials discussed in this article.

Supplementary Appendices

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