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Acanthamoeba keratitis therapy: time to cure and visual outcome analysis for different anti-amoebic therapies in 227 cases

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Competing interest statement:
Vincenzo Papa is an employee of SIFI S.p.A. who manufacture and supply PHMB in Italy, and who are carrying out studies to develop it as a licenced therapy for the treatment of *Acanthamoeba* keratitis in Europe. The remaining authors have no proprietary or commercial interest in any materials discussed in this article.

Contributorship:
Vincenzo Papa designed the study with input from Darwin Minassian and John Dart; Paolo Rama provided access to patients in Milan; Cherry Radford reviewed the notes and prepared the data for analysis; Darwin Minassian tabulated the data and carried out the statistical analysis; John Dart prepared the manuscript with input from all the other authors.

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Synopsis

Treatment outcomes were evaluated for 227 Acanthamoeba keratitis patients. PHMB 0.02% monotherapy for the initial treatment of AK is as effective as biguanide+diamidine combination therapy. The outcome data are the most detailed available.

Abstract

Aims

To test the hypothesis that *Acanthamoeba* keratitis (AK) outcomes differ for different topical anti-amoebic therapies (AAT) and to provide detailed patient outcome data.

Methods

A retrospective cohort study of 227 patients developing AK between 25/07/1991-10/08/2012. Inclusion criteria required a complete record of AAT treatment for both the primary outcome of a medical cure rate at 12 months and the secondary outcome of Snellen VA ≤6/24 and/or surgical intervention. Analysis used multivariable regression to control for differences in baseline disease characteristics for both primary and secondary outcomes with unadjusted analyses for other outcomes. Subjects were categorised for analysis both by the AAT used at baseline and also by mutually exclusive AAT (patients exposed to all the drugs in each group, and no others, for some period). AAT categories were PHMB monotherapy, PHMB+diamidine, PHMB+chlorhexidine+diamidine, diamidine monotherapy and Other AAT.

Results

Analysis by baseline AAT showed no notable difference between treatments for both a medical cure at 12 months in 60.79% (138/227) or for a poor outcome in 49.34% (112/227). When AAT were analysed by mutually exclusive groups PHMB monotherapy provided the best outcomes. These findings are subject to bias requiring careful interpretation. Overall cure rates for the 214 subjects with resolved outcomes were 94.27% (214/227), median time to cure 5 months (interquartile range 3.25-9.00 months) and range 1-26.24 months.

Conclusion

PHMB 0.02% monotherapy for the initial treatment of AK is as effective as biguanide+diamidine combination therapy. Chlorhexidine monotherapy was too infrequent for comparison. The outcome data are the most detailed available.

Introduction

Acanthamoeba is one of the most severe causes of keratitis, resulting in maybe the most prolonged and severe morbidity of any of the corneal infectious diseases[1]. Successful treatment requires eradication of the biocidal resistant encysted form of the organism as well as the much more susceptible trophozoite [2, 3]. Evidence for the efficacy of all drugs in current use for AK has been comprehensively reviewed[4]. The most widely studied are the biguanides and diamidines[4, 5]. The formulation of treatment guidelines is limited by lack of supportive data; only one randomised controlled trial[6] and 17 published case series with some outcome data (of which only 7 reported 50-128 patients)[5, 7, 8]. These studies provide only low level evidence for evaluating the effects of these drugs[9]. Additional evidence for the selection of amoebicidal drugs, on the basis of *in vitro* data, is unreliable because of the lack of a standard methodology and an uncertain relationship to *in vivo* outcomes.[4, 10-12] Lastly, the experience of clinicians and clinical scientists in managing the disease is often limited because of its rarity. In 2010, amongst corneal specialists in the USA, biguanides were the most widely used drugs as monotherapy, and a biguanide with a diamidine as dual therapy[13]. Current recommendations from national organisations for first line treatment from the Centers for Disease Control and Prevention in the USA (2017) and from the Royal College of Ophthalmologists in the UK (2013) are the same; these advise treating with PHMB 0.02% or chlorhexidine 0.02%, either as monotherapy or with the addition of a diamidine [14, 15].

This study was expanded from a dataset needed to inform a current randomised controlled treatment trial.[16]

Our aims were to test our hypothesis that there are differences between the outcomes of treatment for different anti-amoebic therapies (AAT). This was done for the different AAT by comparing (i) the clinical cure rates at 12 months without surgery (the primary outcome measure) (ii) the proportions of patients with poor outcomes (the secondary outcome measure), using both unadjusted analyses and a multivariable analysis to adjust for potentially confounding differences in baseline disease severity, and (iii) unadjusted analyses of time to a cure (with or without surgery), poor outcomes and severe

visual loss both overall and for each individual AAT at the end of treatment. The definitions of the terms used above are given in the Methods section.

Methods

The study was approved by the Moorfields Eye Hospital Clinical Research Management and Audit Department, and by the San Raffaele Hospital Ethics Committee.

Definitions used in this study

- *Cure*: clinical evidence of elimination of *Acanthamoeba* - an intact corneal epithelium with no clinical signs of ocular inflammation after discontinuing AAT for 30 days. This included 2 patients who required an enucleation to cure the disease.
- *Medical cure rate within 12 months*: a cure without the need for surgery, independent of visual acuity (including blindness).
- *Poor outcome*: final visual acuity ≤6/24 and/or a need for surgery.
- *Severe visual loss*: visual acuity (≤3/60), no perception of light or enucleation.
- *Baseline*: is the date of initiation of AAT. Any AAT added within 24 hours of starting the first AAT was also considered as Baseline AAT
- *Baseline AAT*: the AAT started at baseline.
- *Mutually exclusive AAT*: patients in any one AAT treatment group must have been exposed to all the drugs in that group, and no others, for at least some time during their treatment (this might have been only a few days) and could only be established at the end of treatment.
- *Other AAT*: any single drug or drug combination used to treat <25 subjects were combined for analysis in this grouping.
- *Adjusted analyses*: adjusted for baseline characteristics using multivariable regression with adjustment for confounding by differences in baseline characteristics.
- *Unadjusted analyses*: were without adjustment for confounding by baseline characteristics.
- *Disease staging* and *Disease severity*:

- *Stage 1 AK* - corneal epitheliopathy only.
- *Stage 2 AK* - the presence of one or more corneal epithelial defects, perineural infiltrates or stromal infiltrate, in addition to Stage 1 findings.
- *Stage 3 AK* - disease a corneal ring infiltrate, and one or more features of Stage 2 disease.
- *Scleritis* and *hypopyon* were also recorded.
- *Severe AK disease* was defined as scleritis and/or hypopyon and/or Stage 3 disease.

- *The 100-subject dataset*: a 100 patient subset in whom severe disease episodes developing after baseline were recorded. This was not possible for the whole dataset. This additional data was collected for regulatory purposes to obtain approval for the design of our current randomised controlled trial.[16] The additional data included the onset of severe AK disease and/or stromal infiltrates that developed after the initiation of AAT throughout the full course of treatment.

Patient identification, inclusion and exclusion criteria and data collection parameters

Those treated for AK at Moorfields Eye Hospital (London, UK) and San Raffaele Hospital (Milan, Italy) between 25/07/1991 and 10/08/2012, and with retrievable medical records. Only patients with a diagnosis of AK, for whom the primary and secondary outcome measures could be ascertained from complete data were included in the analysis. Diagnostic criteria for AK included the following: a positive *Acanthamoeba* culture; histopathological confirmation of trophozoites and/or cysts; culture-negative cases having *Acanthamoeba* cysts on confocal microscopy; and patients without any of the foregoing who had a keratitis with perineural corneal infiltrates and/or ring infiltrates and/or a clinical course consistent with AK and a response to AAT. Patients with concurrent bacterial keratitis were included, as well as those developing bacterial keratitis as a complication of AK. Patients with a diagnosis of other causes of chronic microbial keratitis, including fungal and herpes keratitis were excluded. The medical records of all patients included in the study were used to collect these data: age, gender, ethnic group, year of diagnosis, keratitis treatments given before baseline, the delay from

symptom onset to the delivery of AAT, presence of scleritis and/or hypopyon and/or stage 3 disease at baseline, each AAT or combination of AAT used, the changes in these throughout the disease course and the clinical outcomes in terms of clinical cure and vision.

Anti-Acanthamoeba therapy classification

All patients were treated with topical AAT which included the two biguanides (chlorhexidine 0.02% or PHMB 0.02%) and one of two diamidines (either propamidine 0.1% or hexamidine 0.1%) either as monotherapy or in combinations. The two diamidines were not distinguished from each other for analysis both being categorised as diamidines. Subjects were categorised for analysis according to which treatments were received and was done in two ways: by baseline AAT and by mutually exclusive AAT ; the definitions for these are given above. This classification resulted in four groups of drugs for analysis which were different for the two methods of categorisation. Within each of these two categories patients using adjunctive anti-amoebic, or potentially anti-amoebic drugs, for any period were recorded.

Endpoints evaluated

For each of the different AAT groups we measured both the medical cure rates within 12 months, the rates for patients with a poor outcome and the rates of those with severe visual loss. The analyses for the medical cure within 12 months and a poor outcome were both unadjusted and adjusted for confounding factors. Lastly, and to provide an assessment of the outcomes of current treatments, we carried out a Kaplan-Meier survival analysis for ongoing inflammation (Kaplan-Meier plots) and estimated cure rate statistics (median, interquartile range, minimum and maximum cure time) both for any treatment, and for the different AAT treatment groups. These analyses were replicated for each method of categorising the subjects by AAT. The analysis by baseline AAT is presented here, and that by mutually exclusive AAT in Supplementary Appendix 1.

Statistical analyses

Statistical analyses were performed using Stata software version 14 (StataCorp LP, College Station, TX). Unadjusted analysis of bivariate data, and a multivariable analysis with adjustment for confounding, were carried out for both the measure of the medical cure rate at 12 months and for the visual acuity outcomes of all patients cured, including those requiring surgical therapy, at any time point after the start of AAT.

Unadjusted analysis of bivariate data

Outcome proportions were estimated for the various AAT groups with 95% confidence intervals using binomial exact procedures. The outcome proportions were also calculated for other baseline exposures known or suspected to be potential confounders. The association between mode of AAT and outcome of AK were evaluated using Poisson regression with robust standard errors and an offset to obtain estimates of % success ratios or failure risk ratios (RR) and p-values, without adjustment for confounders. In this preliminary analysis, the association between outcome and other independent variables (potential confounders) were assessed using the Fisher's exact test.

Adjusted analysis - multivariable regression with adjustment for confounding

Multivariable Poisson regression models with robust standard errors and an offset were constructed for estimation of RR for comparisons of medical cure rates without surgery by 12 months for the different groups of AAT and for comparing the risk of a sub-optimal visual outcome. The first step in the model building process was to include in the initial model: (i) the exposure variable of primary interest (AAT mode), (ii) all the variables (potential confounders) with p-values <0.2 from the preliminary assessment of associations with the outcome, and (iii) all the variables known/thought *a priori* to be risk factors for poor outcome of AK. The latter were: age; severity of disease at baseline (presence of hypopyon and/or scleritis and /or Stage 3 disease); and corticosteroid use prior to the start of AAT). The second step was to use stepwise procedures (algorithms) for inclusion/exclusion of variables, with instruction to keep the variable of primary interest, and with monitoring of the effects of an exclusion on the RR estimates of primary interest, so as not to exclude a variable for gain in precision if it was a confounder, e.g. if its exclusion materially altered the RRs of primary interest.

The aim was to obtain a *valid* estimate of RRs even at the expense of losing some precision in its estimation.[17]

Results

Data was extracted from the medical records of 232 patients meeting the inclusion criteria for having complete data from the onset of symptoms, and for both primary and secondary outcome measures. However, for 5/232 the follow-up periods were less than 31 days which was was considered too short for clinical resolution to have had a reasonable chance of occurring and these 5 were excluded from analysis leaving the 227 patients who were included in the study. Of these 227 patients 177 were from Moorfields represents circa 41% of the approx. 430 cases seen at Moorfields in the study recruitment period .[18]

Supplementary Table 1 summarises the demographic data of patients at the initiation of AAT. No patients were included who had concurrent fungal or herpes keratitis although over 44% of patients were treated with anti-viral and steroid therapy before the diagnosis of AK due to misdiagnosis or delayed diagnosis of AK. Concurrent or misdiagnosed bacterial keratitis was also common (over 66%). Over 34% of patients had severe disease (scleritis and/or hypopyon and/or ring abscess).

Table 1 describes the patients categorised by the baseline AAT given (that prescribed at the time of diagnosis of AK). These were PHMB+diamidine, PHMB monotherapy, Diamidine monotherapy, and Other AAT (including groups with <25 individuals). This table also lists events after the initiation of AAT for each group. These include (i) *switching of baseline therapy* to an alternative AAT (ii) *the use of additional adjunctive potentially anti-amoebic drugs* (oral voriconazole and itraconazole) or *topical PHMB 0.06%* which are listed in a footnote for each group and (iii) the proportion of patients in each group requiring *oral anti-inflammatory and/or immunosuppressive therapy* for the management of scleritis.[19] *Switching of baseline therapy*: was highest at 88% (22/25) for diamidine monotherapy. By contrast the PHMB+diamidine baseline AAT had only 24.6% (28/114) of subjects switching. The use of *additional adjunctive potentially anti-amoebic drugs* (oral voriconazole and itraconazole) or *topical*

Table 1
Baseline anti-amoebic treatment (AAT) [AAT given at diagnosis] categories for 227 patients with *Acanthamoeba* keratitis. Baseline AAT groups (n<25) were combined into the Other AAT group. PHMB and chlorhexidine were both used at a 0.02% concentration, except where stated in the footnotes. The diamidines (propamidine 0.1% and hexamidine 0.1%) were categorised together. Within each group there were small numbers of patients receiving additional potentially anti-amoebic drugs; these are listed in the footnotes for each group. The proportion of patients in each group requiring oral anti-inflammatory and/or immunosuppressive therapy for the management of scleritis are shown.

Baseline AAT group			**Change of AAT after baseline**	**Adjunctive anti-inflammatory treatment introduced after baseline**			
	n	**%**	**n (%) switching to an alternative AAT**	**Oral immuno-suppressants n (%)**	**Oral immunosuppressants used n (%)**		
					Steroids[5]	**Non-steroidals[6]**	**Both steroids & non-steroidals**
PHMB+Diamidine[1]	114	50.2	28 (24.6%)	13 (11.40)	8 (7.02)	None	5 (4.39)
PHMB monotherapy[2]	50	22.0	24 (48.0%)	13 (26.00)	2 (4.00)	3 (6.00)	8 (16.00)
Diamidine monotherapy[3]	25	11.0	22 (88.0%)	6 (24.00)	3 (12.00)	1 (4.00)	2 (8.00)
Other AAT groups combined [4]: Diamidine+chlorhexidine (n=21); Chlorhexidine monotherapy (n=14); PHMB+chlorhexidine (n=1); PHMB+chlorhexidine+diamidine (n=2)	38	16.74	20 (52.6%)	8 (21.05)	6 (15.79)	None	2 (5.26)
Total	**227**	**100**	**94 (41.4%)**	**40 (17.62)**	**19 (8.37)**	**4 (1.76)**	**17 (7.49)**

Numbers of subjects [percent] in each AAT group given PHMB 0.06% or anti-fungal drugs after baseline:
1. Oral voriconazole (n 1) [0.88%], oral itraconazole (n 6) [5.26%], oral voriconazole & itraconazole (n 1) [0.88%], topical PHMB 0.06% (n 3) [2.56%]
2. Oral voriconazole (n 1) [2.00%], oral itraconazole (n 2) [4.00%], oral voriconazole & itraconazole (n 2) [4.00%], topical PHMB 0.06% (n 6) [11.76%]
3. Oral itraconazole (n 2) [8.00%], topical PHMB 0.06% (n 1) [3.85%]
4. Oral itraconazole (n 4) [10.53%], oral voriconazole & itraconazole (n 1) [2.63%], topical PHMB 0.06% (n 4) [10.53%]

Steroid and non-steroid immunosuppressive drugs given:
5. Prednisolone, methylprednisolone
6. Methotrexate, azathioprine, mycophenolate, ciclosporin

PHMB 0.06% was similar for each group. The proportion of subjects in each baseline AAT requiring *oral anti-inflammatory and/or immunosuppressive therapy* was lower for PHMB+diamidine than that for the other baseline AAT. The implications of these potentially confounding events occurring after the initiation of treatment are described in the Discussion.

Supplementary Table 1 describes the frequency distribution for the 14 baseline factors that were considered for inclusion in the multivariable analyses. Table 2 compares the medical cure rate at 12 months for the four different AAT groups for all 227 patients. Both the unadjusted comparison, and the comparison adjusted for confounding factors are shown and compared with the results for PHMB monotherapy. A medical cure at 12 months (the primary outcome measure) was achieved in 60.79% (138/227) cases; there was no difference of note between the different AAT, either unadjusted or adjusted for confounding by baseline characteristics (cure rate ratios close to 1.0, & overall p-values: 0.817 & 0.528 respectively). The two right-hand columns show overall cure rates at 12 months for patients requiring surgery and also for those who had no surgery and failed to resolve by 12 months; there was no significant difference between AAT for these outcomes (overall Chi square p=0.304 – see footnote in Table 2). The absence of any difference in outcomes between AAT, in terms of overall cure rates with or without the need for surgery, is more easily appreciated by the descriptive analysis in Supplementary Table 2 which includes additional information not in Table 2.

Table 2

Comparison of cure rates within 12 months of initiating *Acanthamoeba* keratitis treatment for 227 patients using different baseline anti-amoebic therapies (AAT). Unadjusted comparison, and comparison adjusted for confounding factors, for a medical cure without surgery. Outcomes for those patients without a medical cure at 12 months are also given.

Baseline AAT	Primary Outcome for MEDICAL CURE RATE comparisons within 12 months of starting AAT for groups defined by baseline AAT compared to PHMB monotherapy						Outcomes for patients not achieving a medical cure at 12 months without surgery	
			Unadjusted		Adjusted for confounding [1]			
	Cure	% Cure (95% CI)	% Cure ratio [2] (95% CI)	p-value [3]	% Cure ratio [2] (95% CI)	p-value [3]	Cure rate at 12 months for medical therapy with surgery n (%)	Failure to cure at 12 months n (%)
#2 PHMB monotherapy (***Referent***)	29/50	58.00 (43.21-71.81)	**1.00 (*Referent*)**		**1.00 (*Referent*)**		8/50 (16.00)	13/50 (26.00)
#1 PHMB, Diamidine	70/114	61.40 (51.83-70.37)	1.06 (0.80-1.40)	0.687	1.00 (0.78-1.29)	0.999	13/114 (11.40)	31/114 (27.19)
#3 Diamidine monotherapy	15/25	60.00 (38.67-78.87)	1.03 (0.69-1.54)	0.868	1.07 (0.79-1.45)	0.642	6/25 (24.00)	4/25 (16.00)
#7 Other AAT [4]	24/38	63.16 (46.00-78.19)	1.09 (0.78-1.53)	0.623	1.09 (0.79-1.50)	0.602	9/38 (23.68)	5/38 (13.16)
Totals	**138/227**	**60.79 (54.11-67.19)**		0.817 [5]		0.528 [5]	**36/227 (15.86)**	**53/227 (23.35)**
#1 + #3 +#7 combined	109/177	61.58 (53.99-68.78)	1.06 (0.82-1.38)	0.656	1.03 (0.81-1.31)	0.804	28/177 (15.82)	40/177 (22.60)

1. Adjustment made for the confounding effect of the following baseline factors affecting outcomes (see Supplementary Table 1): age, year of diagnosis, severity of disease at baseline (presence of hypopyon and/or scleritis and /or Stage 3 disease), and corticosteroid use prior to the start of AAT. Further adjustment for "Delay in starting AAT" in the model made no material difference to the risk ratios reported for these initial AAT groups.
2. Probability of success in the baseline AAT group divided by probability of success in PHMB monotherapy as the referent. Values >1.0 indicate a higher success rate compared to the Referent.
3. p-values and confidence intervals are from Poisson regression with robust standard errors
4. Diamidine+chlorhexidine (n=21); chlorhexidine monotherapy (n=14); PHMB+chlorhexidine (n=1); PHMB+chlorhexidine+diamidine (n=2)
5. p-values testing the null hypothesis (Ho): no association between AAT groups & the binomial outcome "cured without surgery; yes, no".

Test of the Ho: no association between AAT groups & the trinomial outcome "cured without surgery, cured with surgery, not cured", p=0.304

Table 3 compares poor outcomes (defined as visual acuity ≤6/24 and/or surgical intervention) for 227 subjects. Overall 49.34% (112/227) had a poor outcome; there was no difference between the AAT groups either unadjusted or adjusted for baseline characteristics. The two right hand columns show the proportions of patients with both severe visual loss (Snellen ≤ 3/60) in 24.67% (56/227) and no light perception (including 2 enucleations) in 2.20% (5/201).

Table 3.
Comparison of poor outcomes (defined as visual acuity ≤6/24 and/or surgery) with both unadjusted comparison and comparison adjusted for potentially confounding differences in baseline characteristics for 227 patients grouped by their baseline anti-amoebic therapies (AAT). In addition, the two right hand columns give the unadjusted comparisons for these AAT for patients with severe visual loss (≤ 3/60) or no light perception.

Baseline AAT	Poor outcomes for different baseline AATs with PHMB monotherapy as referent						Severe vision loss	
	Numbers	Percent (95% CI)	Unadjusted		Adjusted for confounding [1]		Snellen acuity ≤ 3/60 n (%)	No light perception n (%)
			Risk Ratio [2] (95% CI)	p-value [3]	Risk Ratio [2] (95% CI)	p-value [3]		
#2 PHMB only (***Referent***)	22/50	44.00 (29.99-58.75)	**1.00 (*Referent*)**		**1.00 (*Referent*)**		11/50 (22.00)	1/50 (2.00)
#1 PHMB + Diamidine	58/114	50.88 (41.35-60.36)	1.16 (0.81-1.66)	0.431	1.28 (0.91-1.82)	0.155	31/114 (27.19)	1/114 (0.88)
#3 Diamidine only	11/25	44.00 (24.40-65.07)	1.00 (0.58-1.72)	>0.999	0.91 (0.58-1.44)	0.692	4/25 (16.00)	2/25 (8.00)
#7 Other AAT [4]	21/38	55.26 (38.30-71.38)	1.26 (0.82-1.92)	0.293	1.34 (0.87-2.06)	0.180	10/38 (26.32)	1/38 (2.63)
Totals	**112/227**	**49.34 (42.66-56.03)**					**56/227 (24.67)**	**5/227 (2.20)**
#1 + #3 +#7 combined	90/177	50.85 (43.24-58.43)	1.16 (0.82-1.63)	0.412	1.23 (0.89-1.71)	0.216	45/177 (25.42)	4/177 (2.26)

1. Adjustment made for the confounding effect of the following baseline factors affecting outcomes (see Table 3 for detail): age, year of diagnosis, severity of disease at baseline (presence of hypopyon and/or scleritis and /or Stage 3 disease), and corticosteroid use prior to the start of AAT. Further adjustment for delay from onset to starting AAT made no material difference to the risk ratios reported for these baseline AAT groups.
2. Estimated as risk of failure (numerator) compared to that for PHMB monotherapy (denominator).
3. p-values and confidence intervals are from Poisson regression with "robust" standard errors.
4. Diamidine+chlorhexidine (n=21); chlorhexidine monotherapy (n=14); PHMB+chlorhexidine (n=1); PHMB+chlorhexidine+diamidine (n=2)

Supplementary Table 3 describes the proportions of patients in each AAT group developing inflammatory complications after the initiation of AAT to the end of treatment in the 100 subset dataset of patients (the subset for whom we have these data). A higher proportion of patients on PHMB monotherapy developed these compared to those subjects using other AAT.

Figure 1 includes the Kaplan-Meier curves for the time-to-cure (equivalent to survival of inflammation over time) for the 227 patients independent of visual outcome or the need for surgical intervention, both overall and for subjects categorised by baseline AAT. The Kaplan-Meier curves were closely packed together and criss-crossed, suggesting no differences of note between any of the AAT therapies categorised in this way. Overall 25% of subjects were cured within 3.25 months of diagnosis and 25% required more than 9 months to achieve a cure with some subjects taking up to 26 months. The median time to cure was 5 months. Thirteen patients who failed to achieve cure by the time of their last visit were included in the survival analysis, but not in estimation of time-to-cure.

Supplementary Appendix 1 describes this same analysis but carried out on the subjects categorised by mutually exclusive AAT. The results and discussion are included in the Appendix. In brief PHMB monotherapy was associated with significantly better medical cure rates at 12 months at 84.62% (22/26) compared to PHMB+diamidine and PHMB+chlorhexidine+diamidine as well as a lower rate of poor outcomes in 19.23% (5/26) compared to these combinations and the shortest median time to an overall cure.

Discussion

This is the largest series of outcomes of AK patient treatments reported to date. The study evaluates the most widely used and recommended AAT[4, 13, 16] although there were too few subjects using chlorhexidine 0.02% monotherapy for this to be analysed as a single group. The study provides both a comparison of the efficacy of these AAT and detailed outcomes of treatment for AK.

The patient cohort includes only those with complete records of their whole disease course and excludes those referred without a complete history of drug use, those discharged back to local care, and those lost to follow-up. The Moorfields cohort of 177 represented circa 41% of the total number seen in the study period. This selection criterion may have introduced some bias some bias towards the selection of more severely affected patients. For our analysis of outcomes for different AAT it has been possible to control for the baseline characteristics at the initiation of AAT. Unfortunately, there are other potential confounding factors for which we cannot control in a retrospective study and which limit the conclusions that can be drawn from the analyses presented here. These confounders have resulted in different results depending on how the subjects were categorised. When categorised by AAT given at the initiation of therapy there was no difference in the outcomes between them. This statement requires qualification for which some data is in Table 1. *Switching of baseline therapy*: was highest at 88% (22/25) for diamidine monotherapy which reflects the practice patterns of the centres referring patients to Moorfields and San Raffaele where diamidine monotherapy is often prescribed at diagnosis, usually because biguanides are not stocked in their hospitals; almost all of these patients had a biguanide added on referral to our tertiary care centres. By contrast the PHMB+ diamidine baseline AAT included AAT switching in only 24.6% (28/114) of subjects, the lowest proportion, which reflects both the addition of a biguanide to a diamidine in the diamidine monotherapy group and the practice patterns of many of the 13 Consultants at both centres who use biguanide and diamidine combination therapy as their baseline AAT. It is important to note that the use of a diamidine with a biguanide does not imply that patients were maintained on the diamidine throughout the course of treatment. We were not able to measure the time for which each patient was maintained on each drug but diamidines are often discontinued at our centres, because of concerns about both efficacy and toxicity, whereas patients were kept on a biguanide throughout treatment. The use of *additional adjunctive potentially anti-amoebic drugs* (oral voriconazole and itraconazole) or *topical PHMB 0.06%* was similar for each group and unlikely to have affected any differences in group comparisons. Table 1 shows that the proportion of subjects in each baseline AAT group requiring *oral anti-inflammatory and/or immunosuppressive therapy* was lower for PHMB+ diamidine than for the other baseline AAT and significantly lower than that for PHMB monotherapy (exact p=0.034) which could reflect the beneficial effects of the combination therapy and the ineffectiveness of PHMB monotherapy in modifying their onset. However, we think this is unlikely

given that this conclusion is not supported by (i) the findings for PHMB monotherapy which was the most effective AAT in the analysis of mutually exclusive AAT, which are discussed below and for which the data is given in Supplementary Appendix 1, and (ii) because of the higher proportion of subjects switching to different therapies after starting PHMB monotherapy compared to PHMB+diamidine (Table 1) which means that we cannot be sure which drugs these subjects were using at the time that they developed severe inflammatory complications.

When subjects were categorised by mutually exclusive AAT (Supplementary Appendix 1) then PHMB monotherapy, when given as initial therapy after diagnosis and not switched to alternative AAT, was associated with both the best medical cure rates in 12 months and the best visual outcomes compared to patients treated in the other AAT groups. Analysis of the 100 subject dataset showed that this outcome was unlikely to have been confounded by the development of severe inflammatory complications during the curse of treatment. There are however other potentially confounding factors and a full discussion of these is in Supplementary Appendix 1.

The outcome data provided by this study are the most detailed available for a large series of patients. One of the most useful results of our analysis is the outcome data for a medical cure, overall cures and visual outcomes for this patient cohort. These are valuable for advising patients of potential outcomes, for clinicians and clinical researchers for comparison with their own results, and for public health and research funding organisations who need these data to understand the substantial burden imposed by this orphan disease. Our data on visual outcomes is comparable with some of that provided in previously published studies. In a review of 15 previous case series published in 2009[5], outcomes for the 4 case series[20-23] reporting the highest numbers of patients or eyes (between 36 and 105) with visual outcomes and/or treatment success data, comparable to those reported in this study, showed good outcomes in over 73% and loss of all useful vision and enucleation in up to 12% (the latter figure from the only study using propamidine with no biguanide)[23]. All of these studies, with the exception of two patients in one study[22] were using biguanides or diamidines as monotherapy or in combination therapy[5]. Of the two subsequent comparable publications one describes 128 cases with visual outcome data of Snellen VA ≥ 20/25 in 69%

(50.66% in our study) and Snellen VA ≤20/200 in 9% with 7% progressing to multiple keratoplasties or enucleations; although the treatment regimens were not described the corresponding author has stated that chlorhexidine 0.02% or 0.06% monotherapy was most commonly used, sometimes in combination with propamidine.[8] The second study describes 59 patients (59 eyes) treated with combination therapy using triple therapy with PHMB 0.02%, propamidine, and an antibiotic, in whom 16/51 (31.37%) had VA ≤20/100 after treatment (49.34%) in our study[7]. For our analysis by mutually exclusive AAT the PHMB monotherapy results, although not directly comparable because of different VA outcome levels, are as good or better than those in the previous studies. For the other AAT groups in our study the proportion of patients with vision ≤3/60 or worse was probably worse than those previously reported despite the use of similar AAT regimens; the reasons for this are unclear.

Do our findings inform clinicians about AAT treatments that might provide better results? One conclusion from analysis by baseline AAT, without consideration of the confounding factors, might be that PHMB monotherapy, PHMB+diamidine or PHMB+chlorhexidine+diamidine could be equally efficacious or, from our analysis using mutually exclusive AAT that PHMB monotherapy might be the best therapy. Although we cannot comment on chlorhexidine 0.02% monotherapy it is probably similarly effective to PHMB 0.02%.[6] However, we do not think that diamidine monotherapy should be used because the minimal cysticidal concentrations vary widely[4, 24] with high values reported for both propamidine and hexamidine in clinically resistant isolates. [25, 26] This is as opposed to the biguanides which have shown more consistent cysticidal activity, in which clinical resistance has not related to *in vitro* resistance,[24, 27] and for which toxic reactions are probably less frequent, leading to their current choice as first line AAT.[4, 15, 16]

Given the long treatment courses and poor outcomes for AK when treated with topical biguanides and topical diamidines, as reported in this study and all others, there has been a search for other drugs that might be more successful. These include azoles (most commonly voriconazole), miltefosine and caspofungin amongst others[4]. *In vitro* results for the cysticidal activity of voriconazole have given very disparate results, with some *in vitro* reports showing good activity, and other *in vitro* studies showing limited or absent cysticidal activity. Clinical outcomes for AK, treated with oral or topical voriconazole, are described for only

10 cases, in 6 case series. There is even less data for the *in vitro* cysticidal activity, or for clinical outcomes, for these other drugs[4]. Currently there is no evidence that therapies, other than biguanides, are better; despite the recognition that our current drug formulations and/or treatment protocols leave much to be desired.

This study was initiated to inform a current Phase III study comparing PHMB 0.08% monotherapy to propamidine and PHMB 0.02% combination therapy[16]. Here we provide evidence that PHMB 0.02% monotherapy is as effective as other widely used AAT. Because monotherapy is simpler to administer and less costly than combination therapy with a diamidine these findings support the use of PHMB monotherapy as a first line treatment for AK. Whether PHMB monotherapy will reduce progression of disease after the start of treatment cannot be answered adequately in a retrospective study design of this type. However, if the current randomised controlled trial successfully completes[16], we can expect an answer to this question.

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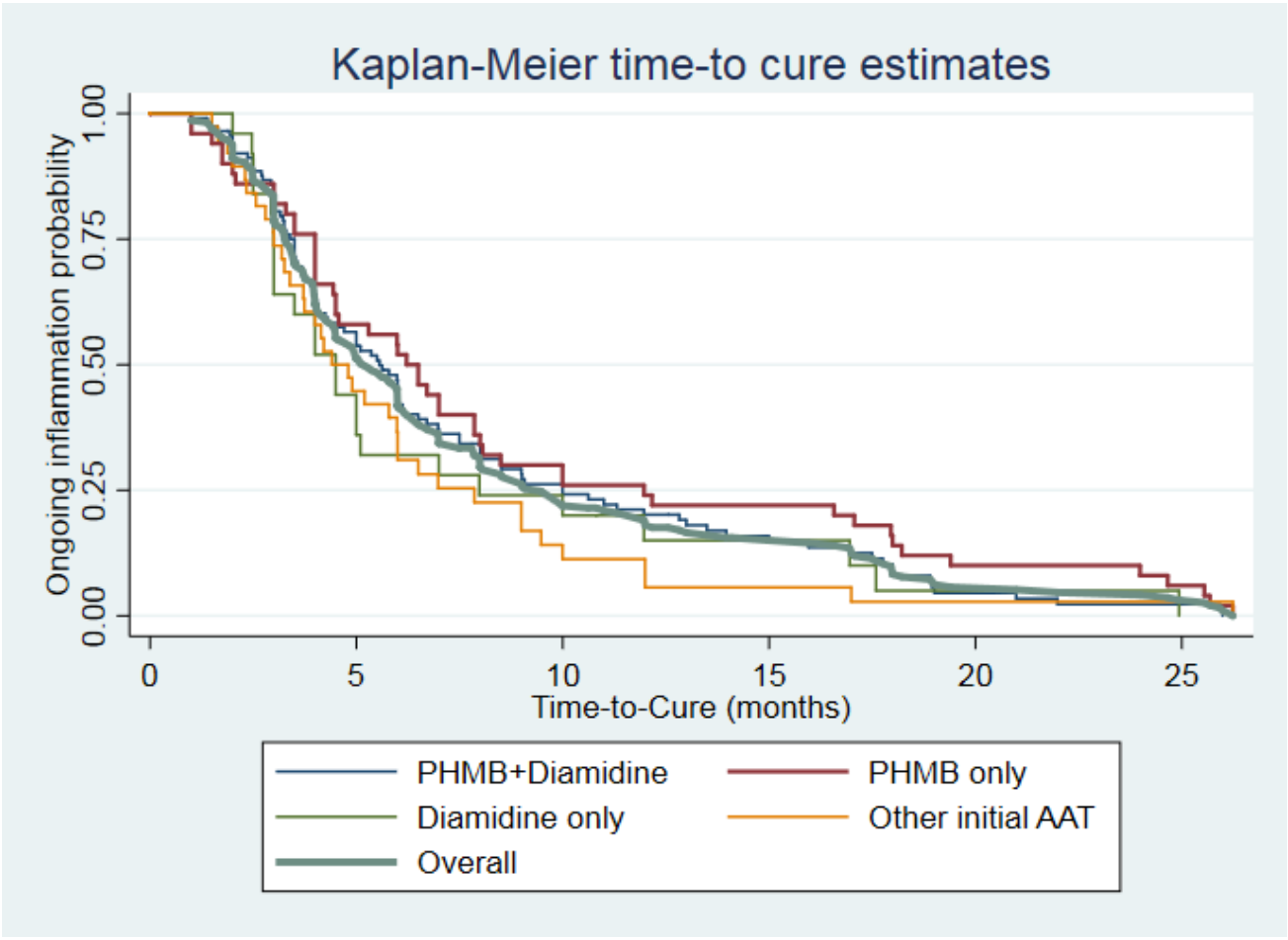
Figure 1 legend

Figure 1 includes the Kaplan-Meier curves for the time-to-cure (equivalent to survival of inflammation over time) for 227 patients independent of visual outcome or the need for surgical intervention, both overall and for subjects categorised by baseline AAT. The table shows estimates of time-to-cure. Thirteen patients who failed to achieve cure by the time of their last visit were included in the survival analysis, but not in the table showing estimates of time-to-cure. Two subjects having extreme values (outliers) for time-to-cure were identified. These were retained in the analysis, but the data adjusted using an established statistical procedure; without this correction the K-M plots would be misleading.

Figure 1



Initial AAT	N	Cure n (%)	Median months to cure	IQR (25th-75th percentiles)		Minimum - Maximum months	
PHMB, Diamidine	114	103 (90.35)	5.10	3.25	9.01	1.00	25.99
PHMB monotherapy	50	50 (100.00)	6.36	4.00	11.97	1.00	26.24
Diamidine monotherapy	25	24 (96.00)	4.25	3.00	7.49	2.00	24.93
Other AAT *	38	37 (97.37)	4.41	3.00	6.98	1.50	26.24
Totals	**227**	**214 (94.27)****	**5.00**	**3.25**	**9.00**	**1.00**	**26.24**

* Other AAT: diamidine, chlorhexidine (n=21); chlorhexidine monotherapy (n=14); PHMB, chlorhexidine (n=1); PHMB, chlorhexidine, diamidine (n=2)

** 13 patients were excluded from the final analysis of cure having unresolved outcomes. These 13 were included in the rest of the study tables as they met the criteria for the primary and secondary outcomes and had visual acuity recorded at their last visit

Supplementary Table 1
Frequency distribution of baseline characteristics in relation to the primary outcome measure of having a medical cure of *Acanthamoeba* keratitis, within 12 months of starting anti-amoebic therapy (AAT), without requiring surgery.

Characteristics at Baseline	Frequency		Medical cure within 12 months[1]			
	n	%	Failure	Success	% Success	p-value
Age:						
mean {sd}: 35.70 {13.78}						
median {IQR}: 33 {25 - 44}[2]						
Age group:						
13-33	118	51.98	29	89	75.42	< 0.001
34-76	109	48.02	60	49	44.95	
Gender:						
Male	100	44.05	42	58	58.00	0.494
Female	127	55.95	47	80	62.99	
Ethnic group:						
Caucasian	135	78.95	54	81	60.00	0.705
Other	36	21.05	13	23	63.89	
Unknown	56		22	34	60.71	
Year of Diagnosis:						
1991-2000	38	16.74	22	16	42.11	0.011
2001-2012	189	83.26	67	122	64.55	
Delay in starting AAT -in days[3]:						
mean {sd}: 45.60 {49.27}						
median {IQR}: 30 {14 - 56}[2]						
<=30days	114	52.29	33	81	71.05	0.005
>30days	104	47.71	50	54	51.92	
Unknown	9		6	3	33.33	
Scleritis &/or Hypopyon:						
Absent	183	83.94	59	124	67.76	< 0.001
Present	35	16.06	23	12	34.29	
Unknown	9		7	2	22.22	
Stage-3 disease:						
Absent	149	72.68	46	103	69.13	0.001
Present	56	27.32	32	24	42.86	
Unknown	22		11	11	50.00	

Supplementary Table 1 (continued)

Characteristic at Baseline	Frequency		Clinical cure within 12 months[1]			
	n	%	Failure	Success	% Success	p-value
Advanced or Severe disease:						
Absent	144	65.45	39	105	72.92	< 0.001
Present [4]	76	34.55	45	31	40.79	
Unknown	7		5	2	28.57	
Corticosteroids pre-AAT:						
No	126	55.51	36	90	71.43	< 0.001
Yes	101	44.49	53	48	47.52	
Antivirals pre-AAT:						
No	125	55.07	36	89	71.20	0.001
Yes	102	44.93	53	49	48.04	
Antibiotics pre-AAT:						
No	76	33.48	34	42	55.26	0.251
Yes	151	66.52	55	96	63.58	
Oral anti-fungals pre-AAT	1/227 on itraconazole					
Study Centre:						
Milano (Ospedale San Raffaele)	46	20.26	18	28	60.87	> 0.999
London (Moorfields Eye Hospital)	181	79.74	71	110	60.77	

1. Success defined as a medical cure within 12 months without surgery.
2. Inter-quartile range: presented as the 25th and 75th percentiles of the data.
3. One patient diagnosed at 330 days
4. Presence of scleritis or hypopyon or Stage-3 disease

Supplementary Table 2
Cure (clinical resolution) within 12 months of baseline (start of anti-amoebic therapy)

This descriptive table gives additional information on the requirement of surgery and total cure rates at 12 months not included in **Table 2.**

Baseline anti-amoebic therapy (AAT)	No. of Patients	Number having surgery n (%)	Cure without surgery n (%)	Cure with surgery n (%)	Total cure n (%)
PHMB+diamidine	114	33 (28.95)	70 (61.40)	13 (11.40)	83 (72.81)
PHMB monotherapy	50	14 (28.00)	29 (58.00)	8 (16.00)	37 (74.00)
Other AAT (including diamidine monotherapy) *	63	22 (34.92)	39 (61.90)	15 (23.81)	54 (85.71)
Totals	227	69 (30.40)	138 (60.79)	36 (15.86)	174 (76.65)

* Diamidine alone (n=25); diamidine+chlorhexidine (n=21); chlorhexidine monotherapy (n=14); PHMB+chlorhexidine (n=1); PHMB+chlorhexidine+diamidine (n=2)

Supplementary Table 3

For analysis of subject by baseline anti-amoebic therapy (AAT).

Proportions of patients in the 100 subject dataset developing new inflammatory complications (corneal stromal infiltrates (including all ring abcesses), hypopyon, or scleritis/limbitis) in each of the 4 anti-amoebic (AAT) groups, during follow-up after the initiation of AAT

Initial AAT group	**Number**	**Developed stromal infiltrates, hypopyon, or scleritis/limbitis during follow-up after start of AAT**	
		Number	Percent (%)
PHMB+Diamidine	56	21	37.50
PHMB monotherapy	19	14	73.68
Diamidine monotherapy	5	0	0.00
Other AAT *	19	7	36.84
Total **	99	42	42.42

* Diamidine+chlorhexidine (10), chlorhexidine monotherapy (8), PHMB+diamidine+chlorhexidine (1)

** One patient with missing data excluded.

Supplementary Appendix 1

Analysis of the study population categorised by mutually exclusive anti-amoebic therapy (AAT) as opposed to the analysis that is presented in the body of the paper, in which subjects are categorised by the baseline AAT used, the analysis in this Appendix categorises the subjects by mutually exclusive AAT. This means that patients in any one group must have been exposed to all the drugs in that group, and no others, for at least some time during their treatment. The analysis of outcomes using this method of categorising patients by AAT used has relevance to the conclusions of the study. It shows that for patients who stayed on the same therapy (either monotherapy or multiple drug therapy) throughout treatment that PHMB monotherapy had better outcomes than the other AAT that we evaluated. Both of the methods used for categorising subjects for analysis by AAT are confounded by changes of therapy during the course of treatment. Insight into potentially confounding factors affecting outcomes can be derived from the proportions of patients developing new inflammatory complications after the initiation of therapy for both analyses. For the analysis of subjects by baseline AAT the switching of drugs after starting treatment shows differences that affect the interpretation of the results. For this analysis the proportions of subjects who switched drugs in each group is inappropriate because the definition resulted in no switching in the PHMB monotherapy group; because of this the switching analysis has been omitted from the analysis for this method of categorising subjects by mutually exclusive AAT. Detailed results are provided below including a more detailed discussion of its limitations.

Results

Supplementary Appendix Table 1 describes the patients categorised by the 4 principal mutually exclusive AAT's used for their treatment: these were PHMB+diamidine, PHMB+chlorhexidine+ diamidine, PHMB monotherapy and Other AAT groups combined. Within each group there were small numbers of patients receiving additional potentially adjunctive anti-amoebic drugs; these are listed in a footnote for each group. The proportion of patients in each group requiring oral anti-inflammatory and/or immunosuppressive therapy for the management of scleritis are shown. The PHMB+chlorhexidine+diamidine group, followed by those on PHMB+diamidine (25.9%) included higher proportions of patients on adjunctive anti-amoebic therapy and requiring treatment with oral anti-inflammatories/immunosuppressants. The latter, with oral itraconazole, are used for management of scleritis[19].

Supplementary Table 1 (common to the analyses of subjects in both categories of AAT used in this study) describes the frequency distribution for the 14 baseline factors that were considered for inclusion in the multivariable analysis in relation to the cure rate without surgery at 12 months. Supplementary Appendix Table 2 compares the medical cure rate without surgery at 12 months for the four different AAT groups for all 227 patients. Both the unadjusted comparison and the comparison adjusted for confounding factors are shown and compared with the results for PHMB monotherapy. PHMB monotherapy had the highest clinical cure rate at 12 months of 84.62% (22/26) and was used as the referent for comparison with the other AAT. Adjusted comparisons show that PHMB monotherapy was significantly better than PHMB+chlorhexidine+diamidine combination therapy as well as to all the other comparative treatments combined. For all comparator AAT groups combined (those apart from PHMB monotherapy) the medical cure rate at 12 months was 57.7% (116/201) a further 15.92% (32/201) were cured within 12 months but with the additional requirement for surgical therapy and 26.36% (53/201) were not cured within 12 months. The two right-hand columns show overall cure rates at 12 months for patients requiring surgery which was highest in the PHMB+chlorhexidine+diamidine and Other AAT groups. Failure to cure by 12 months was between 22% and 42% in the PHMB+diamidine and in the PHMB+chlorhexidine+diamidine groups respectively.

Supplementary Appendix Table 3 describes those subjects with poor outcomes for each AAT. Overall 49.34% (112/227) had a poor outcome. PHMB monotherapy had the smallest proportion of subjects with a poor outcome 19.23% (5/26) which was significantly better than that for all other groups except Other AAT in both unadjusted and adjusted analyses.

Supplementary Appendix Table 4 describes the proportions of patients in each AAT group developing inflammatory complications after the start of AAT (in the period following baseline to the end of treatment) for the 100-subject dataset for whom these data were available. There were no significant differences between AAT groups for this. There was no evidence that the observed incidence of complications in the PHMB monotherapy group was lower compared to the PHMB+ diamidine group or the Other AAT groups combined and was only slightly lower than that in the PHMB+chlorhexidine+diamidine group.

Supplementary Appendix Figure 1 describes the overall time to a cure, independent of visual outcome or the need for surgical intervention, for AAT categories. For PHMB monotherapy cures were achieved within 12 months whereas for PHMB+diamidine and PHMB+diamidine+chlorhexidine the overall time to a cure required over 26.24 months and, for the two outliers, up to 37 months.

Discussion

This analysis (by mutually exclusive AAT) has shown that PHMB monotherapy, when given as initial therapy after diagnosis and not switched to other AAT (n 26), was associated with both the best cure rates without surgery within 12 months (84.62%), and the best visual outcomes (80.77% for Snellen >6/24) without surgery, as well as the lowest proportion of patients with severe vision loss (3.85%) at the end of treatment, compared to patients treated in the other AAT groups.

The results also show that for AAT, other than PHMB monotherapy, more adjunctive anti-amoebic drugs were used, and that a higher proportion of these patients required either oral anti-inflammatory or immunosuppressive therapies, used for the treatment of scleritis. These differences can be interpreted in several ways: (1) as a result of these other AAT being less effective and therefore resulting in more severe inflammatory complications, (2) as an effect of clinician's attempts to reduce a toxic response to medications by switching treatments, (3) as a clinician attempt to try and reduce the time taken for the infection to resolve, or (4) because higher proportions of the patients using AAT other than PHMB monotherapy developed severe inflammatory complications, unrelated to their baseline characteristics or treatment. We have investigated the possibility of (4) in the 100-subject dataset, for whom the onset of inflammatory events after starting AAT was documented (Supplementary Appendix Table 4 below); there was no difference in the proportions of patients in each AAT group who developed these episodes. However, it is not possible in a retrospective study like this, even with these detailed data regarding the onset of severe inflammatory complications developing after the start of AAT, to distinguish whether these are definitively related to the inefficacy or side effects of the initial baseline AAT, or to some other currently unidentified cause. This question will be best answered in a prospective randomised controlled trial of PHMB monotherapy versus combined AAT therapy, such as the one that is currently in progress[18].

Supplementary Appendix Table 1
Anti-amoebic treatment (AAT) categories for 227 patients.

Subjects were categorised by mutually exclusive AAT: patients in any one AAT treatment group must have been exposed to all the drugs in that group, and no others, for at least some time during their treatment (this might have been only a few days) and could only be established at the end of treatment. PHMB and chlorhexidine were both used at a 0.02% concentration, except where stated in the footnotes. The diamidines (propamidine 0.1% and hexamidine 0.1%) were categorised together. The treatment groups are mutually exclusive: each subject is only in one group. Within each group there were small numbers of patients receiving additional potentially anti-amoebic drugs; these are listed in the footnotes for each group. The proportion of patients in each group requiring oral anti-inflammatory and/or immunosuppressive therapy for the management of scleritis are shown.

AAT group	**n**	**%**	**Oral anti-inflammatories or immunosuppressants n (%)**	**Oral immunosuppressants used n (%)**		
				Steroids[5]	**Non-steroidals**[6]	**Both steroids & non-steroidals**
PHMB+diamidine[1]	116	51.1	14 (12.07)	7 (6.03)	2 (1.72)	5 (4.31)
PHMB+chlorhexidine, diamidine[2]	63	27.8	22 (34.92)	8 (12.70)	2 (3.17)	12 (19.05)
PHMB monotherapy[3]	26	11.5	1 (3.85)	1 (3.85%)	None	None
Other AAT groups combined[4]: chlorhexidine+diamidine (n 8) PHMB+chlorhexidine (n 3) chlorhexidine monotherapy (n 8) diamidine monotherapy (n 3)	22	9.7	3 (13.64)	3 (13.64%)	None	None
Total	**227**	**100**	**40 (17.62)**	**19 (8.37%)**	**4 (1.76%)**	**17 (7.49)**

Numbers of subjects [percent] in each AAT group given PHMB 0.06% or anti-fungal drugs after the start of AAT
1. Oral voriconazole (n 1) [0.86%], oral itraconazole (n 2) [1.72%], PHMB 0.06% (n3) [2.58%]
2. Oral voriconazole (n 2) or oral and topical voriconazole (n 3) [7.93%], oral itraconazole (15) [23.80%], PHMB 0.06% (n 11) [17.46%]
3. Oral itraconazole (n 1) [3.84%]
4. None

Steroid and non-steroid immunosuppressive drugs
5. Prednisolone, methylprednisolone, dexamethasone, betamethasone
6. Methotrexate, azathioprine, mycophenolate, cyclosporin

Supplementary Appendix Table 2
Comparison of medical cure rates within 12 months of initiating *Acanthamoeba* keratitis treatment for 227 patients using mutually exclusive anti-amoebic therapies (AAT).

Unadjusted comparison, and comparison adjusted for confounding factors, for a medical cure without surgery at 12 months. Outcomes are also given for those patients not being cured at 12 months without surgery.

AAT group	MEDICAL CURE RATE comparisons, within 12 months of starting anti-amoebic treatment, for different AAT groups compared to PHMB monotherapy						Outcomes for patients not achieving a medical cure at 12 months without surgery	
	Cure	% Cure (95% CI)	Unadjusted		Adjusted for confounding [1]		Cure rate for medical therapy with surgery at 12 months	Failure to cure at 12 months
			% Cure ratio [2] (95% CI)	p-value [3]	% Cure ratio [2] (95% CI)	p-value [3]	n (percent)	n (percent)
PHMB monotherapy	22/26	84.62 (65.13-95.64)					4/26 (15.38)	0
Referent Comparators:								
PHMB+diamidine	77/116	66.38 (57.02-74.88)	1.27 (1.03-1.57)	0.023	1.25 (1.03-1.53)	0.026	13/116 (11.21)	26/116 (22.41)
PHMB+chlorhexidine+ diamidine	23/63	36.51 (24.73-49.60)	2.32 (1.61-3.34)	<0.001	2.13 (1.50-3.30)	<0.001	13/63 (20.63)	27/63 (42.85)
Other AAT[4]	16/22	72.73 (49.78-89.27)	1.16 (0.86-1.58)	0.330	1.10 (0.83-1.44)	0.513	6/22 (27.27)	0
Totals	**138/227**	**60.79 (54.11-67.19)**					**36/227 (15.86)**	**53/227 (23.34)**
All comparators combined	116/201	57.71 (50.56-64.63)	1.47 (1.20-1.79)	<0.001	1.40 (1.15-1.69)	0.001	32/201 (15.92)	53/201 (26.36)

1. Adjustment made in the final model for the confounding effect of the following baseline factors affecting outcomes (see Table 3 for detail): age, year of diagnosis, severity of disease at baseline (presence of hypopyon and/or scleritis and /or Stage 3 disease), and corticosteroid use prior to the start of AAT. Delay in starting AAT made no material difference to the risk ratios reported for these AAT groups.
2. Probability of success in PHMB monotherapy divided by probability of success in the comparator AAT as the referent.
3. p-values and confidence intervals are from Poisson regression with robust standard errors
4. Chlorhexidine+diamidine (n=8), PHMB+chlorhexidine (n=3), chlorhexidine monotherapy (n=8), diamidine monotherapy (n=3)

Supplementary Appendix Table 3
Comparison of poor outcomes for 227 subjects using mutually exclusive anti-amoebic therapies (AAT).

Unadjusted comparison, and comparison adjusted for confounding factors, for suboptimal visual outcome (defined as final visual acuity ≤6/24 AND/OR surgical intervention). The two right hand columns show the proportion of subjects having severe vision loss at the end of treatment for each AAT group.

AAT grouping	Poor outcomes for different AATs with PHMB monotherapy as referent						Severe vision loss	
	Numbers	Percent (95% CI)	Unadjusted		Adjusted for confounding [1]		Snellen acuity ≤ 3/60 n (%)	No light perception n (%)
			Risk Ratio [2] (95% CI)	p-value[3]	Risk Ratio [2] (95% CI)	p-value [3]		
PHMB monotherapy (***Referent***)	5/26	19.23 (6.55-39.35)					1/26 (3.85)	0
PHMB+diamidine	54/116	46.55 (37.24-56.05)	2.42 (1.07-5.46)	0.033	2.29 (1.04-5.02)	0.039	22/116 (18.97)	2/116 (1.72)
PHMB+chlorhexidine+diamidine	44/63	69.84 (56.98-80.77)	3.63 (1.62-8.13)	0.002	3.22 (1.46-7.08)	0.004	28/63 (44.44)	3/63 (4.76)[5]
Other AAT[4]	9/22	40.91 (20.71-63.65)	2.13 (0.83-5.43)	0.114	2.00 (0.79-5.06)	0.143	5/22 (22.73)	0
Totals	**112/227**	**49.34 (42.66-56.03)**					**56/227 (24.67)**	**5/227 (2.20)**
All comparators combined	107/201	53.23 (46.08-60.29)	2.77 (1.24-6.16)	0.013	2.55 (1.18-5.51)	0.017	55/201 (27.36)	5/201 (2.49)

1. Adjustment made in the final model for the confounding effect of the following baseline factors affecting outcomes (see Table 2 for detail): age, year of diagnosis, severity of disease at baseline (presence of hypopyon and/or scleritis and /or Stage 3 disease, and corticosteroid use prior to the start of AAT. Delay in starting AAT made no material difference to the risks ratios reported for these AAT groups.
2. Estimated as risk of failure (numerator) compared to that for PHMB monotherapy (denominator)
3. p-values and confidence intervals are from Poisson regression with robust standard errors
4. Chlorhexidine+ diamidine (n=8), PHMB+chlorhexidine (n=3), chlorhexidine monotherapy (n=8), diamidine monotherapy (n=3)
5. Including 2 Enucleations

Supplementary Appendix Table 4

Proportions of those in the 100-subject dataset developing new inflammatory complications (corneal stromal infiltrates (including all ring abscesses), hypopyon, or scleritis/limbitis) in each of the 4 mutally exclusive anti-amoebic (AAT) groups, during follow-up after the start of AAT.

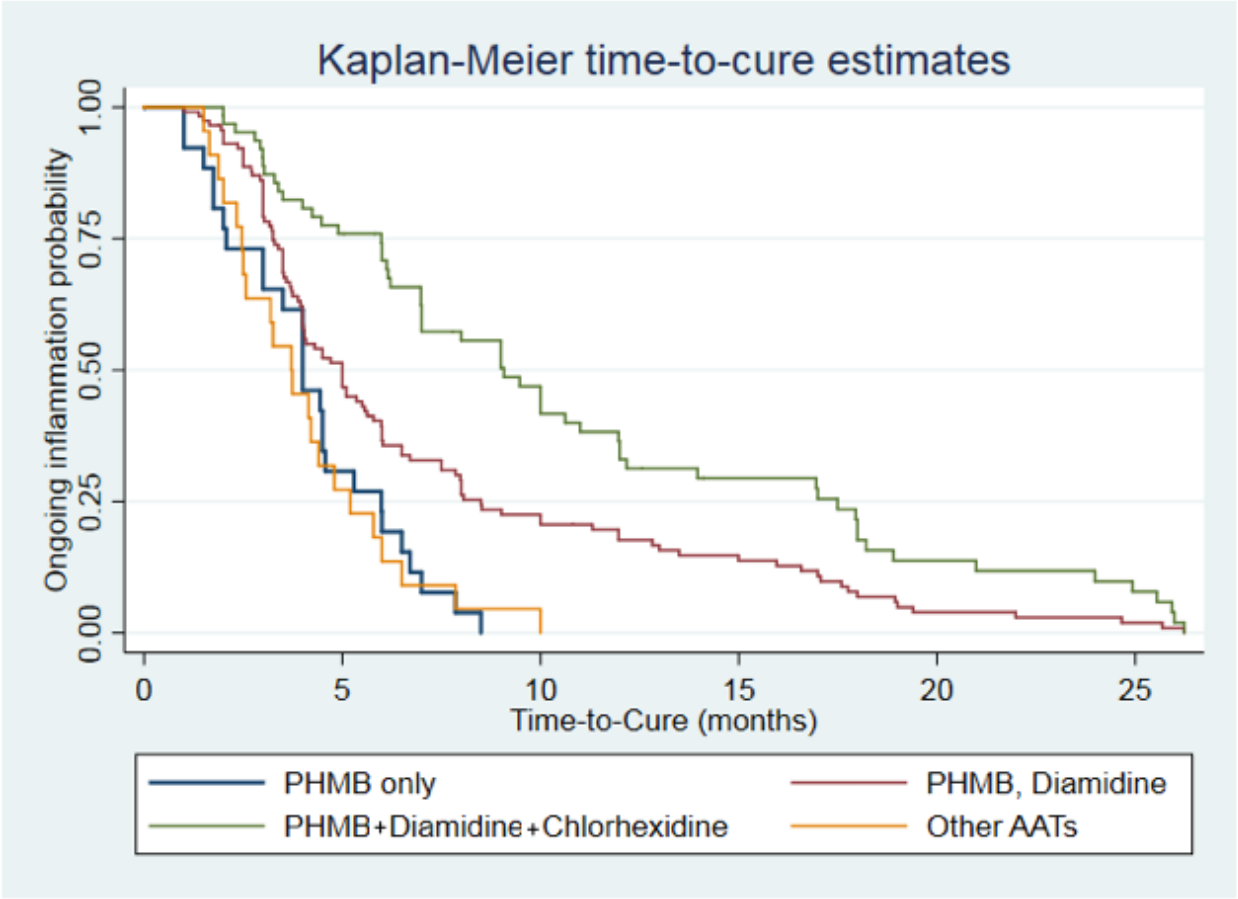
AAT group	**Number**	**Developed stromal infiltrates, hypopyon, or scleritis/limbitis during follow-up after start of AAT**	
		Number	Percent (%)
PHMB monotherapy	7	4	57.14
PHMB, Diamidine	53	19	35.85
PHMB, Diamidine, Chlorhexidine	24	15	62.50
Other AAT groups combined*	15	4	26.67
Total**	99	42	42.42

* Diamidine monotherapy (n=1), chlorhexidine monotherapy (n=7), chlorhexidine, diamidine (n=7)

** One patient with missing data excluded

Supplementary Appendix Figure 1

This Figure includes the Kaplan-Meier curves for the time-to-cure (equivalent to survival of inflammation over time) for 227 patients independent of visual outcome or the need for surgical intervention, both overall and for subjects categorised by **mutually exclusive AAT**. The table shows estimates of time-to-cure. Thirteen patients who failed to achieve cure by the time of their last visit were included in the survival analysis, but not in the table showing estimates of time-to-cure. Two subjects having extreme values (outliers) for time-to-cure were identified. These were retained in the analysis, but the data adjusted using an established statistical procedure; without this correction the K-M plots would be misleading.



AAT group	N	Cure n (%)	Median months to cure	IQR (25th-75th percentiles)		Minimum - Maximum months	
PHMB only	26	26 (100.00)	4.00	2.07	5.98	1.00	8.50
PHMB, diamidine	116	109 (93.97)	4.70	3.25	8.00	1.00	26.24
PHMB, chlorhexidine, diamidine	63	57 (90.48)	9.00	4.90	16.95	1.99	26.24
Other AAT*	22	22 (100.00)	3.73	2.47	5.19	1.50	9.99
Totals	**227**	**214 (94.27)**	**5.00**	**3.25**	**9.00**	**1.00**	**26.24**

* Chlorhexidine, diamidine (n=8), PHMB, chlorhexidine (n=3), chlorhexidine monotherapy (n=8), diamidine monotherapy (n=3)

** 13 patients were excluded from the final analysis of cure having unresolved outcomes. These 13 were included in the rest of the study tables as they met the criteria for the primary and secondary outcomes and had visual acuity recorded at their last visit.