Can Clinical Signs of Trachoma Be Used after Multiple Rounds of Mass Antibiotic Treatment to Indicate Infection?

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PURPOSE. To evaluate combinations of clinical signs, using a modified World Health Organization (WHO) grading scheme, to predict a very low prevalence of infection at the community level after at least three rounds of mass drug administration (MDA).

METHODS. Seventy-one villages had three to seven rounds of MDA. A random sample of 7828 children ages 5 years and younger was evaluated for trachoma, and determination of *Chlamydia trachomatis* infection was made. Proportions of children positive for infection were evaluated against all possible combinations of follicular trachoma (TF) and inflammatory trachoma (TI). High-risk signs (HRS) for infection were defined as those indicating the infection prevalence was >20%. The sensitivity and specificity of prevalence of HRS in identifying communities with infection was examined.

RESULTS. The median community infection prevalence was 3.8% interquartile range (IQR) (1.8%, 7.7%); the median trachoma prevalence was 9.4%, IQR (6.6%, 15%). Severe TI, combination of TF and TI (WHO criteria), or severe TF with signs of inflammation were predictive of infection in the community, but the absence of these HRS was not an indicator of low infection rates.

CONCLUSIONS. The use of HRS to determine the infection status of a community was not useful in predicting whether MDA could be stopped. (*Invest Ophthalmol Vis Sci.* 2011;52:8806–8810) DOI:10.1167/iov.11-8074

Trachoma, the result of repeated infection with *Chlamydia trachomatis*, is the leading infectious cause of blindness.¹ The World Health Organization (WHO) strategy for trachoma control consists of surgery for trichiasis, antibiotics for reduction of the infectious pool in the community, and facial and

environmental hygiene to interrupt transmission. In endemic communities the community load of active trachoma, and infection, resides in preschool children.^{2,3} Simple assessment of the clinical signs of follicular trachoma is deemed sufficient to determine the communities and districts that require trachoma control interventions. Mass drug administration (MDA) of endemic districts and communities with azithromycin is recommended for at least 3 years if the prevalence of trachoma is 10% or greater in children ages 1 to younger than 10 years, followed by resurvey to determine whether further MDA is warranted. A recent change in guidelines suggests that when the prevalence is high at baseline, such as 30%, there is no advantage to a resurvey before 5 years of MDA.

Antibiotics target the infectious agent, *C. trachomatis*, not the clinical signs of trachoma. Prevalence surveys have repeatedly shown that the rates of clinical signs of trachoma, follicular trachoma (TF), and inflammatory trachoma (TI) in children are higher than the actual rates of infection.^{4–9} These findings are expected, as shown in animal models and longitudinal studies in which follicles in the conjunctiva take a long time to resolve after infection has disappeared.^{10–13} Using the WHO simplified grading scheme for trachoma assessment, approximately 50% to 90% of those with TI and 30% to 50% of those with TF have concomitant infection,¹⁴ with even lower rates after MDA.^{8,11,15,16} Even if free of infection, communities with TF prevalence ≥10% will continue another series of rounds of MDA in compliance with WHO guidelines.

However, the other sign of active trachoma, TI, appears to be more sensitive to antibiotic treatment.¹⁵ If a combination of more or less severe signs of trachoma would better capture the infection status, then the use of such a combination might be useful to identify communities in which infection is either absent or persists. Although studies have examined the relationship between clinical signs of trachoma and infection in individual children, there has not been a systematic evaluation of the use of clinical signs, and especially an expanded array of clinical signs, at the community level to help determine whether infection persists after MDA.

As part of a larger survey of trachoma and infection in Tanzanian communities that had at least three annual rounds of mass treatment, we expanded the WHO simplified grading scheme to include additional levels of TF and TI that could be graded reliably. The aim of this study was to determine whether a combination of these clinical signs could predict the presence, or absence, of infection at the community level and could have programmatic utility.

METHODS

Communities

Details on the selection of communities have been described in detail elsewhere.^{11,16} In brief, communities in Tanzania were eligible for this

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TABLE 1. Expanded Classification of Trachoma Based on WHO Simplified Grading Scheme

Sign	None (grade 0)	Mild (grade 1)	WHO Grading Scheme Minimum Definition (grade 2)	Severe (grade 3)
Follicular trachoma (follicles at least 5 mm in size)	0 follicles	1–4 follicles	5–10 follicles	>10 follicles
Intense trachoma	No deep tarsal vessels obscured	Inflammatory thickening and ≤50% of deep tarsal vessels obscured	Inflammatory thickening and >50% of deep tarsal vessels obscured	No vessels visible because of inflammatory thickening

study if they had baseline data on trachoma rates before the implementation of the Tanzania National Trachoma Control Program and data from the National Trachoma Control Program on mass treatment coverage with azithromycin. Additionally, eligible communities had to have received at least three rounds of mass treatment: 71 communities with three to seven rounds of MDA were enrolled. Before study initiation, community leadership provided consent for overall community participation in the study; individual consent was obtained before ocular examination. All procedures and protocols were approved by the Johns Hopkins University Institutional Review Board and the National Institute for Medical Research in Tanzania.

Sentinel Children

A sentinel sample of 100 to 120 children ages 5 and younger was randomly selected from a census of each community. The age group 5 years and younger was chosen because the baseline survey in all these villages was conducted on a random sample of children ages 5 years and younger before WHO guidelines specifying the selection of children younger than 9 years. In any case, the age group 5 and younger is at greater risk for both infection and clinical disease.

Data Collection

Ocular Assessment. Both upper eyelids of each sentinel child were examined. A senior trachoma grader (HM) performed all trachoma assessments in the field using our expansion of the WHO simplified grading scheme¹⁷ (Table 1). In the expanded grading scheme, TF grade 1 is mild, with <5 follicles measuring 0.5 mm each, and goes to TF grade 3, which is severe with >10 follicles; the WHO grade of TF includes grades 2 (5–10 follicles) and 3. Similarly, TI grade 1 is mild inflammation with <50% of deep tarsal vessels obscured, and TI grade 3 is severe with all vessels obscured by inflammation. The WHO grade of TI includes grades 2 and 3 for TI. An ocular photograph using a Nikon (Tokyo, Japan) D-series camera and a 105-mm 1:2.8-G lens (Micro; Nikon) was taken of every child. For this study, the field grade was used because it would be used in practically any programmatic setting.

Ocular Specimen. A laboratory assistant flipped the left upper eyelid of each child. Using a swab (Dacron; Fisher Health Care, Houston, TX) held two-thirds of the way down the shaft, the senior grader rubbed the eyelid three times, twirling the swab as it crossed the tarsal plate. The swab was then inserted into a vial without touching the edge or the outside of the vial. The vials were placed in a cold box in the field, transferred each evening to a freezer, and stored until shipped frozen in a dry state to the Johns Hopkins International Chlamydia Laboratory for processing.

Specimens were processed with the Roche Amplicor *C. trachomatis* qualitative PCR assay from Roche Molecular Systems (Indianapolis, IN) according to strict protocol outlined in the manufacturer's kit directions. Procedures are summarized as follows: Each swab was eluted by vortexing in lysis buffer (Amplicor CT/NG; Roche) in polypropylene tubes, and specimen diluent (Amplicor) was added. Using a known positive sample in the laboratory, positive and negative *C. trachomatis* (CT) processing controls were created; two CT+ and two CT− processing controls were run with each batch of specimens.

After the hybridization step, detection was accomplished by measuring the optical density at A_{450} . The assay result for the negative controls should be $<0.2 A_{450}$, and the assay result for the positive controls should be $\geq 0.8 A_{450}$ for ocular specimens for a valid run. Samples in valid runs with values $\geq 0.8 A_{450}$ were counted as positive, and samples $<0.2 A_{450}$ were counted as negative. Samples for which the result was equivocal (≥ 0.2 , <0.8) were retested; if equivocal twice, they were left as equivocal and called not positive in the analyses because no run was ≥ 0.8 .

Quality Assurance

Detailed protocols were followed during the ocular examinations, including the wearing of sterile gloves that were changed after testing of each child. At least five control field swabs were taken of the air in each village, and all were all negative.

To assess the reliability of the expanded WHO grading scheme for our grader, a set of 60 photographs was randomly selected. The field grader read the set twice on separate days and was masked to the previous day's grades. The set represented a reasonable distribution of eyes with trachoma, with 27% TF and 19% TI. The unweighted κ statistic for intraobserver agreement using the expanded scheme was 0.65 for grades of follicular trachoma and 0.78 for grades of trachoma intense.

Statistical Analysis

To examine the association between infection and severity of the trachoma clinical sign, the proportion of children positive for infection

FIGURE 1. Distribution of prevalence of *C. trachomatis* infection and trachoma in the 71 communities.

FIGURE 2. Correlation between follicular trachoma and *C. infection* in the 71 communities.

is presented for all possible combinations of the severity of TF and TI. Children were divided into groups based on all possible combinations of TF and TI. For each combination of clinical signs, the percentage of infected children was calculated. Those combinations in which infection exceeded 20% were considered HRS. We then used the community prevalence of HRS at various thresholds (e.g., 5% or 3%) to classify whether infection in the community was present at $> 2\%$. We chose 2% based on data from Ethiopia suggesting that where infection rates were approximately 3%, re-emergent infection after the cessation of antibiotic treatment occurred.¹⁸ The sensitivity and specificity and the corresponding 95% confidence intervals (CIs) for HRS indicators were calculated. A Lowess smoothing line was used to examine the correlations between follicular trachoma and infection at the community level, and a Spearman correlation coefficient is reported.

RESULTS

The median (IQR) prevalence of follicular trachoma, using the WHO criteria of TF, was 9.4% (6.6%, 15.0%) (Fig. 1). Only one community had no trachoma signs, and 12 communities (16%) had a prevalence below 5%. The maximum trachoma prevalence was 38%. The median prevalence of infection in the 71 communities was 3.8% (IQR: 1.8%, 7.5%) (Fig. 1). Nine communities (13%) had no infection, and seven communities (10%) had minimal infection below 1%. However, 69% of the com-

TABLE 3. Sensitivity and Specificity of Using the High-Risk Combination of Signs to Detect Infection in Children

HRS	Infection Present		
	Yes	No	
Present	120	97	PPV, 55% (48–62)
Absent	309	7291	NPV, 95.9% (95.4–96.4)
	Sensitivity, 28% (24–33)	Specificity, 98.7% (98.4–98.9)	

Values in parentheses are 95% CIs.

munities still had a prevalence of infection $\geq 2\%$, and 44% had a prevalence of infection $\geq 5\%$. A scatterplot shows the relationship between the prevalence of follicular trachoma, using the WHO criteria of TF, and infection (Fig. 2). Of interest, none of the villages with no infection had TF rates $> 10\%$, although many of the villages with infection also had TF rates $< 10\%$. The children were categorized according to the combination of clinical signs of trachoma as described in Table 1. Within each category, the percentage of infected children varied from 2% to 64% (Table 2). The severity of TF and TI increased from none (absence of the minimum follicular or inflammatory signs) to severe (a grade more severe than the minimum WHO criteria). As expected, the infection rate increased as the severity of the clinical signs increased, with the infection rates above 36% in children with severe TI regardless of the presence of TF. The groups at highest risk for infection were those with any severe TI (grade 3), both TF and TI at grade 2, and severe TF (grade 3) with any sign of inflammation (TI grades 1–3). From here on, these clinical signs are referred to as HRS. Of note, though children without signs of trachoma had a prevalence of infection of only 2%, they represented 72% of all children in the study.

The sensitivity and specificity of using HRS to identify a child with infection is shown in Table 3. Although the sensitivity is not high (28%), the specificity of the absence of HRS to indicate the absence of infection is good (97%). However, we wish to use these signs at a community level to indicate infection; to the extent that these HRS cluster in a community, they may or may not be useful to assess the overall community level of infection. Only 10 communities had no HRS. One of the 10 also had no infection, and 3 of 10 had infection $< 1\%$; three communities had infection rates between 1% and 5%, and three had infection rates $> 5\%$. These data suggest that a different approach from the strict absence of HRS as a marker may be more useful.

Thus, we further evaluated the sensitivity and specificity of a community prevalence of HRS of 5% to gauge whether the prevalence of infection was below 2% (Table 4); the specificity was 96%, and the sensitivity was 35%. Despite high specificity,

TABLE 2. Proportions of Children Infected with *C. trachomatis* within Combinations of Clinical Signs of Trachoma

TF	TI			
	None	Mild	WHO Minimum	Severe
None	115/5632* (2.0%)	16/272† (5.9%)	3/35‡ (8.6%)	9/25‡ (36.0%)
Mild	36/739† (4.9%)	46/256‡ (18.0%)	8/42† (19.0%)	12/27‡ (44.4%)
WHO Minimum	23/285† (8.1%)	21/109† (19.3%)	17/36‡ (47.2%)	7/11‡ (63.6%)
Severe	22/145† (15.2%)	50/122‡ (41.0%)	27/44‡ (61.4%)	17/32‡ (53.1%)

* No trachoma, 72%.

† Mild to moderate trachoma, 24%.

‡ Severe trachoma, 4%.

in communities with a prevalence of HRS $<5\%$, most still had a prevalence of infection $>2\%$ (32 of 53 communities). Thus, though the positive predictive value of HRS was strong enough (94%) to identify those communities that still needed treatment, the negative predictive value was poor (40%), and the absence of signs did not mean those villages should have stopped treatment. Adjusting the cutoff prevalence of HRS lower, from 5% to 3%, improved sensitivity but, again, at the expense of specificity (Table 5). Consequently, no combination of HRS and low level of infection suggested a useful approach with programmatic utility.

DISCUSSION

In Tanzanian communities that were baseline hyperendemic for trachoma and had at least three rounds of mass treatment, only 13 (18% of communities) had trachoma $<5\%$, and 69% of the communities still had a prevalence of infection $\geq 2\%$. To improve the performance of clinical signs as a screening tool for communities, this study used a combination of clinical signs (HRS) that were highly associated with the presence of infection in children. Using a prevalence of HRS $\geq 5\%$ as a screen, we correctly identified communities with infection rates $\geq 2\%$ more than 90% of the time. However, the absence of HRS in the community was noninformative because most communities without the signs still had infection rates $\geq 2\%$. The sensitivity of the $\geq 5\%$ cutoff was poor, and, though it improved moderately when the cutoff was lowered to $\geq 3\%$, specificity was lost. In terms of programmatic application, the presence of HRS after MDA offers limited potential toward directing potential MDA cessation.

In this study, we chose a prevalence of infection $>2\%$ as a cutoff for detection. This was based on data from Ethiopia on re-emergent infection even when infection rates were very low after the cessation of MDA.¹⁸ Unfortunately, it is not yet clear at what community level of infection mass treatment can be stopped because the infection will not be sustained or transmitted, and this likely depends on the district prevalences surrounding these communities. For example, a single round of MDA in a community followed by treatment of persons with trachoma with topical tetracycline every 6 months if the baseline level of infection was 9.5% was sufficient to decrease the infection rate to 2.1% after MDA; subsequently, infection declined and never returned after only one more round of MDA.¹² However, the district itself had little trachoma, and this community might have been on the tail end of the disappearance. Other communities—in districts in which trachoma rates were $>10\%$ —with a baseline prevalence of infection at 10% clearly needed more than one round of MDA and, after three or more rounds, still had a prevalence of infection $>2\%$.¹⁶ Although we chose a prevalence of infection $>2\%$ as a cutoff, other values could have been used.

TABLE 4. Sensitivity and Specificity of Using 5% Prevalence of High-Risk Combination of Signs as an Indicator of Infection of $\geq 2\%$ in a Community

HRS	Infection		
	$\geq 2\%$	$< 2\%$	
$\geq 5\%$	17	1	PPV, 94% (73–100)
$< 5\%$	32	21	NPV, 40% (26–54)
	Sensitivity, 35% (22–50)	Specificity, 95% (77–100)	

Values in parentheses are exact 95% CIs for binomial proportions.

TABLE 5. Sensitivity and Specificity of Specific Prevalences of High-Risk Signs (HRS) in Identifying Communities in Which Infection Is $\geq 2\%$

HRS Prevalence (%)	Sensitivity % (95% CI)*	Specificity % (95% CI)*
≥ 3	57 (42–71)	82 (60–95)
≥ 4	41 (27–56)	95 (77–100)
≥ 5	35 (22–50)	95 (77–100)

* Exact 95% CIs for binomial proportions.

This study intended to look at the value of clinical signs of trachoma as potentially indicating an absence of infection after MDA. Thus, the findings apply only to situations in which at least three or more rounds of MDA with azithromycin occurred. It is possible that there is a much tighter correlation of clinical signs with infection before MDA.^{2,4,19} It would be worthwhile to consider whether the HRS have value at the outset of mapping in low-prevalence areas for identifying communities with little or no infection.

A limitation of this study was the use of children ages 5 years and younger while programs use children ages younger than 10 years as sentinel children. We used the younger age group because the main study baseline data were collected in that age group.¹⁶ If we had used the age group younger than 10 years, the prevalence of both infection and HRS would have been lower in the sentinel children, and the number of children with no infection or disease would have been larger because the older children would have had less infection and disease.^{2,6} Thus, the number of cases of HRS and infection in which to carry out our analyses would have been lower, but it is unlikely that our results would have changed. Another potential limitation is the fact that coverage in this program setting was low, with most districts at 75%. If coverage with MDA was higher, the relationship of trachoma signs to infection might have changed. Finally, we recognize that there is uncertainty in estimating the prevalence of infection when it is as low as 2%. Our 95% CI was 0.2% to 6.02%, which means some of these low-prevalence villages could have had infection rates that were actually higher. However, estimates in the villages were measured using clinical signs and infection in the same child. Therefore, even if there was uncertainty about the prevalence from unmeasured children, the correlation was based on what was observed in the children in the sample.

The availability of a test to determine levels of residual community infection would be helpful to assess the need for further treatment; however, no rapid, field-ready laboratory test for *C. trachomatis* exists. For now, trachoma control programs must still rely on TF as the indicator of need for MDA despite the fact that a proportion of those communities are without infection and will, therefore, continue to be treated.

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