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Chlamydial Positivity of Nasal Discharge at Baseline Is Associated with Ocular Chlamydial Positivity 2 Months following Azithromycin Treatment

Emily West Gower[1], **Anthony W. Solomon**[2], **Matthew J. Burton**[2,3], **Aura Aguirre**[2], **Beatriz Muñoz**[1], **Robin Bailey**[2,3], **Martin Holland**[2,3], **Pateh Makalo**[3], **Patrick Massae**[4], **Harran Mkocha**[5], **David C. Mabey**[2], **Sheila K. West**[1]

[1]Dana Center for Preventive Ophthalmology, Johns Hopkins School of Medicine, Baltimore, Maryland [2]London School of Hygiene and Tropical Medicine, London, United Kingdom [3]Medical Research Council Laboratories, Fajara, The Gambia [4]Huruma Hospital, Rombo District, Tanzania [5]Kongwa Trachoma Project, Kongwa, Tanzania

Abstract

Background—Trachoma is the leading infectious cause of blindness. Routes of transmission remain unclear. In this study, the relationship between *Chlamydia trachomatis* Amplicor-positive nasal discharge and Amplicor-positive ocular swabs was investigated (Amplicor; Roche, Indianapolis, IN).

Methods—A longitudinal study was conducted in Tanzania and The Gambia. Eyes were graded for active trachoma; ocular swabs were taken to test for *C. trachomatis*. Children with visible nasal discharge had swabs taken of this material. Participants were offered systemic antibiotics. Two months after treatment, participants were re-examined.

Results—Of the 1128 children participating, 188 (17%) had nasal discharge. Among 188 children with nasal discharge, 64 (34%) nasal swabs were PCR positive. There was a strong correlation between active disease/ocular chlamydial positivity and positive nasal discharge. Children with Amplicor-positive ocular swabs were 9.9 times more likely to have Amplicor-positive nasal discharge than were children without ocular positivity (95% CI: 4.34 – 22.53). Two months after treatment, 16% had an Amplicor-positive ocular swab. Children with positive nasal discharge at baseline were 5.2 times more likely to have an Amplicor-positive ocular swab at 2 months than were children without Amplicor-positive nasal discharge at baseline (95% CI: 1.54 – 17.23), after adjusting for baseline ocular positivity, gender, and study site.

Corresponding author: Emily W. Gower, Dana Center for Preventive Ophthalmology, 116 Wilmer Building, 600 N. Wolfe Street, Baltimore, MD 21287; ewest@jhsph.edu.
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Conclusions—Nasal discharge may provide a source of reinfection with *C. trachomatis*, after antibiotic treatment for trachoma, either through transfer of secretions from nose to eye or from nasal secretions transferred to bed sheets or dirty clothes and back to the eye; alternatively, nasal discharge may be an indicator of severe persistent ocular chlamydial infection that is not cleared with a single dose of antibiotics.

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Trachoma, a disease caused by ocular infection with *Chlamydia trachomatis*, remains hyperendemic in many parts of the world and is the leading infectious cause of blindness worldwide.[1] Although trachoma manifests itself through ocular signs and symptoms, *C. trachomatis* can infect other epithelial surfaces, notably the nasopharynx and gastrointestinal tract.[2] Risk factors for active trachoma have been characterized,[3–8] but routes of transmission are not fully understood.

The World Health Organization has endorsed a strategy known as “SAFE” to help reduce the burden of blinding trachoma worldwide. The components of the SAFE strategy include Surgery to correct inturned lashes, Antibiotics to treat active trachoma, Face-washing to improve hygiene, and Environmental improvements. When face-washing is evaluated within a community, the primary marker for a “dirty” face is the presence of ocular and/or nasal discharge, because this discharge attracts flies, and may be infected. The F component of the SAFE strategy was included based on the results of studies that have shown an association between good personal hygiene (including maintaining a clean face) and lower risk of trachoma.[5,7–10] In a clinical trial of face-washing in Tanzania, West et al.8 demonstrated that having a clean face at two or more follow-up visits was protective against both any trachoma (OR: 0.58; 95% CI: 0.47 – 0.72) and severe trachoma (OR: 0.35; 95% CI: 0.21 – 0.59). In that trial, <25% of children had clean faces at baseline, defined as having no nasal discharge, ocular discharge, or flies on the face. Previous studies have reported the presence of *C. trachomatis* from both nasal and rectal swabs.[2,11] These data suggest that nasal discharge may play a role in trachoma transmission and may result in reinfection.

Data from longitudinal studies conducted in The Gambia and Tanzania provide the opportunity to assess the relationship between positive nasal discharge at baseline and Amplicor-positive ocular swabs 2 months after systemic treatment (Amplicor; Roche, Indianapolis, IN).

Methods

This study was performed in three sites, two in Tanzania and one in The Gambia, each with different levels of trachoma endemicity. In Tanzania, the study was conducted in the subvillage of Maindi, in the Dodoma District of central Tanzania and in the Kahe Mpya subvillage in the Rombo District of northern Tanzania. In The Gambia, the study was conducted in a cluster of 14 small villages in Jareng, Upper Saloum District. Details of the main studies are described elsewhere.[12–16] Briefly, a census was taken of each site, and a survey was conducted of all consenting residents. Each consenting resident underwent an ocular examination to evaluate active trachoma and had a conjunctival swab taken.

In Tanzania, trachoma was graded according to the World Health Organization (WHO) simplified grading scheme,[17] whereas in The Gambia the modified WHO grading scheme

(FPC system [follicles, papillae, cicatrices]) was used.[18] For this report, the FPC system was converted to the WHO-modified grading scheme to provide consistency in grading across sites. Ocular swabs were taken of the tarsal conjunctiva of the right eye (Maindi and Rombo) or left eye (Jareng) of each participant. Children aged less than 9 years with nasal discharge visible outside the margin of the nostril had an additional swab taken of this material. Standard precautions were taken against cross-contamination of samples for different subjects. These precautions were similar across sites, with some slight variations by site. In Rombo, the examiner wore two pairs of latex gloves while examining each subject. Between subjects, he removed the outer pair, sprayed the exterior of the inner pair with alcohol and allowed them to dry before donning another pair. Only the examiner touched the swab, which was snapped off into the collection tube without the use of scissors or a blade. In The Gambia and Kongwa, two individuals worked together to collect the ocular swabs. The examiner everted the lid, while the specimen collector rubbed the Dacron tip across the conjunctiva. The specimen collector snapped off the swab into the tube so that the part that the specimen collector had touched did not enter the tube. Hands were washed between participants. Swabs were stored dry on ice in the field and frozen at −20° within 8 hours of collection, until they were transported to the London School of Hygiene and Tropical Medicine for processing. After examination, all individuals in the village were offered a single dose of oral azithromycin, 20 mg/kg up to a maximum of 1 g.

Ocular and nasal swabs were processed by procedures identical with those described previously.[16] Briefly, once received, laboratory technicians extracted the DNA from the swabs by adding molecular-grade water and vortexing them. A second detergent was then added to finish the preparation of the specimens for analysis. Standard DNA amplification techniques using the Amplicor *Chlamydia trachomatis* qualitative PCR assay were used to analyze the specimens according to the colorimetric assay in the package insert (Roche Molecular Systems, Branchburg, NJ). Equivocal specimens were reassayed and assigned a positive value if optical density results were still between 0.2 and 0.8. Because we cannot be certain whether the detection of *C. trachomatis* DNA in swabs of nasal discharge reflects true *C. trachomatis* infection of the nasopharynx or simply drainage of ocular secretions from an infected conjunctival epithelium via the nasolacrimal duct, we have chosen to refer to a sample of nasal discharge that tested positive for *C. trachomatis* as an “Amplicor-positive nasal discharge” throughout the text. Likewise, low loads of ocular chlamydia do not necessarily translate to biological activity; therefore, we have chosen to be conservative in our description of ocular positivity, referring to swabs as “Amplicor-positive ocular swabs.”

Although all individuals living in the study villages were invited to participate in the study and were offered antibiotic treatment, for the purposes of this report, evaluation was limited to children aged <9 years, as these individuals are the ones from whom swabs of nasal discharge were taken. Frequency tables and Mantel-Haenszel tests for trend were used to evaluate bivariate associations. Logistic regression was used to evaluate the association between ocular positivity and Amplicor-positive nasal discharge at baseline, after adjusting for gender and study site. Multivariate logistic regression also was used to evaluate the association between Amplicor-positive nasal discharge at baseline and ocular positivity 2 months after treatment, after controlling for baseline positivity, gender, and study site. All

study procedures were approved by the Johns Hopkins Joint Committee on Clinical Investigation, the London School of Hygiene and Tropical Medicine and the in-country Institutional Review Boards. The study complied with the tenets of the Declaration of Helsinki.

Results

We examined 1128 children aged <9 years from three villages (Table 1). In this age group, clinically active trachoma prevalence at baseline was highest in Maindi (75.6%) and lowest in Jareng (15.8%). Among all children examined, 188 (16.7%) had fresh, wet nasal discharge at baseline, and a swab was taken of this material. Children with active trachoma were 3.2 times more likely to have nasal discharge than were children without clinical signs of trachoma (95% CI: 2.29–4.39). Children with nasal swabs taken were, on average, younger than those without nasal swabs taken. A total of 64 (34%) nasal swabs were PCR positive. Compared with the village population aged <9 years without nasal discharge, children with nasal discharge were more likely to have active disease (follicular trachoma [TF] and/or trachoma inflammation [TI]) than children with no nasal discharge ($P < 0.0001$).

Active Disease and Nasal Discharge

Among children with nasal swabs taken, a strong association between active disease (TF and/or TI) and positive nasal discharge was noted across all study sites (Table 2). Furthermore, children with intense trachoma inflammation (TI) were more likely to have Amplicor-positive nasal discharge than were individuals with follicular trachoma (TF; $P = 0.035$). For each study site, 50% to 88% of children with nasal discharge and TI had Amplicor-positive nasal discharge, whereas very few children without TF or TI had Amplicor-positive nasal discharge. Among children without signs of active trachoma, the prevalence of Amplicor-positive nasal discharge ranged from 4% to 18% across study sites. The association between active disease and Amplicor-positive nasal discharge was strongest in Maindi, where the highest level of endemicity also was reported.

Ocular Positivity and Nasal Positivity

A similar association was observed between ocular positivity and Amplicor-positive nasal discharge (Table 2). Overall, nearly 60% (13/22) of children with Amplicor-positive ocular swabs also had Amplicor-positive nasal discharge, whereas only 12% (14/115) of children with a negative ocular swab had Amplicor-positive nasal discharge. Again, the association was strongest in Maindi, where trachoma prevalence was highest.

Age and Nasal Positivity

Across all ages studied, there was an association between active disease and Amplicor-positive nasal discharge. The strength of the association between TI and Amplicor-positive nasal discharge increased with age. For children aged <2, 42.9% with TI had Amplicor-positive nasal discharge, whereas 87.5% of children aged 6 to 8 years with TI also had Amplicor-positive nasal discharge. In a logistic regression model predicting Amplicor-positive nasal discharge that included age, TI and the interaction of age and TI, the interaction term was border-line significant ($P = 0.13$). After adjustment for gender and

village, the association between ocular positivity and Amplicor-positive nasal discharge at baseline remained. Children with an Amplicor-positive ocular swab were 9.9 times more likely to have Amplicor-positive nasal discharge than were children with negative ocular swabs (95% CI: 4.34 – 22.53).

Treatment coverage for this population was high. In Maindi, 86% of the total population and 97% of children aged <9 were treated. Similarly, in Rombo, 98% of the entire population and 100% of children aged <9 were treated, and in Jareng, 83% of the entire population and 89% of children aged <9 were treated. Three children from Maindi who had nasal swabs taken at baseline were not treated; in Rombo and Jareng, all children with nasal swabs taken were treated.

Positivity at 2 Months after Treatment

Of the 188 children with nasal discharge at baseline, 169 were examined 2 months after treatment, and 27 (16%) of these children had an Amplicor-positive ocular swab at 2 months (Table 3). In comparison, among the 833 children who did not have a nasal swab taken at baseline but did have an ocular swab taken at follow-up, 64 (7.7%) had an Amplicor-positive ocular swab at 2 months ($P = 0.001$). Of note, we found an association between Amplicor-positive nasal discharge at baseline and ocular positivity at 2 months, even after controlling for baseline ocular positivity. Children with Amplicor-positive nasal discharge at baseline were 5.2 times more likely to have an Amplicor-positive ocular swab at 2 months than were children without Amplicor-positive nasal discharge at baseline after adjustment for gender, study site, and baseline ocular positivity (Table 4).

Fourteen children had Amplicor-positive nasal discharge at baseline but had a PCR-negative ocular swab. Of note, 28% (1/14) of these children had an Amplicor-positive ocular swab at 2 months, whereas only 1 of 90 children without Amplicor-positive nasal discharge and no ocular positivity at baseline had an Amplicor-positive ocular swab at 2 months (Table 5). This rate of positivity was similar to the 2-month positivity rate of children with ocular positivity but without Amplicor-positive nasal discharge at baseline. Children with both Amplicor-positive nasal discharge and ocular positivity at baseline were most likely to have an Amplicor-positive ocular swab at 2 months.

Discussion

C. trachomatis in nasal discharge was common in young children in trachoma-endemic areas, and Amplicor-positive nasal discharge at baseline was associated with ocular positivity at 2 months, independently of baseline ocular positivity. Although the finding of *C. trachomatis*–positive nasal discharge has been reported from Kongwa,[11] this study confirms it in another area of Tanzania and The Gambia. As expected, there was a strong association between trachoma, or ocular chlamydial positivity and Amplicor-positive nasal discharge. West et al.[7] previously demonstrated that in trachoma-endemic areas, approximately 10% of children have constant, severe trachoma, and these children are more likely to advance to scarring. Our data suggest that perhaps these children also are more likely to have more extraocular infection, indicating a failure to clear infection from all sites.

Longitudinal data to determine the risk of ongoing, severe trachoma in this subgroup would confirm this supposition.

A small group of children had an Amplicor-positive ocular swab at 2 months after treatment with azithromycin. We have previously shown in Maindi that such children are more likely to be very young and to have ocular infection at baseline.[13] The present study shows that posttreatment ocular positivity also is related to Amplicor-positive nasal discharge, independent of ocular positivity at baseline. This association may be due to a variety of factors. First, nasal discharge is frequently wiped from the face with a handkerchief or other cloth that also is used to wipe the eyes. If nasal discharge is infected with *C. trachomatis*, it is possible that continual ocular reinfection may occur as a result of self-reinoculation. These children also typically sleep in close quarters on bedclothes or goatskins that are rarely cleaned. Amplicor-positive nasal discharge may then be a source of reinfection created by the individuals themselves or one of their close contacts, such as children sharing the same sleeping space.

Alternatively, the association between Amplicor-positive nasal discharge and ocular positivity after treatment may be explained by heavier extraocular infections among these individuals that are not cleared by the antibiotic regimen (a single dose of 20 mg azithromycin/kg body weight, as recommended by the WHO) used against trachoma in these studies. A study to evaluate the relative effect of higher or multiple doses of azithromycin would be necessary to evaluate this possibility. A further possibility is that this association is due to azithromycin-resistant *C. trachomatis* strains; however, to date no data have demonstrated the emergence of antimicrobial resistance in ocular isolates of this organism.

We recognize that we were testing nasal discharge, which is unlikely to contain epithelial cells; therefore, the positivity that we have noted must come from elementary bodies, not intracellular infection. From our data, we cannot determine whether indeed Amplicor-positive nasal discharge is a true nasopharyngeal infection or whether positivity resulted from drainage of ocular secretions through the nasolacrimal ducts. However, the association between Amplicor-positive nasal discharge at baseline and ocular positivity at 2 months suggests that this discharge may be an important source of transmission or reinfection. As noted by our finding among children without ocular positivity at baseline, children with Amplicor-positive nasal discharge were more likely to have ocular positivity at 2 months.

In summary, we have shown that nasal discharge of children with active trachoma is frequently positive for *C. trachomatis* and that children with an Amplicor-positive nasal discharge are more likely to have an Amplicor-positive ocular swab after treatment than are children with nasal discharge that is *C. trachomatis*–negative or children without nasal discharge. These data suggest that nasal discharge may be a source of trachoma transmission within trachoma-endemic areas through transfer of nasal secretions directly back to the eye or through transfer to bed sheets or clothes and then back to the eye. Alternatively, extraocular infection may be a marker of severe infection that cannot be cleared with a single dose of azithromycin. Continued efforts to implement the F and E components of the SAFE strategy (health education to encourage face-washing, improvements in water supply to facilitate face-washing, and measures to control eye-seeking flies) should be encouraged.

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Table 1
Characteristics of Participants Aged <9 Years with and without Nasal Swabs Taken

	Maindi		Rombo		Jareng		All Study Sites	
	Children without Nasal Discharge	Children with Nasal Discharge	Children without Nasal Discharge	Children with Nasal Discharge	Children without Nasal Discharge	Children with Nasal Discharge	Children without Nasal Discharge	Children with Nasal Discharge
Participants	225	74	271	58	444	56	940	188
Female	120 (53.3)	44 (59.5)	124 (45.8)	30 (51.7)	217 (48.9)	28 (50.0)	461 (49.0)	102 (54.3)
Age (y)								
0.5 to <2	43 (19.1)	11 (14.9)	63 (23.2)	8 (13.8)	106 (23.9)	8 (14.3)	212 (22.6)	27 (13.8)
2 to 3	52 (23.1)	28 (37.8)	57 (21.0)	16 (27.6)	78 (17.6)	25 (44.6)	187 (19.9)	69 (36.7)
4 to 5	41 (18.2)	21 (28.4)	59 (21.8)	20 (34.5)	106 (23.9)	15 (26.8)	206 (21.9)	56 (29.8)
6 to 8	89 (39.6)	14 (18.9)	92 (34.0)	14 (24.1)	154 (34.7)	8 (14.3)	335 (35.6)	36 (19.5)
Trachoma status								
No active disease	61 (27.1)	12 (16.2)	163 (60.2)	21 (36.2)	385 (86.7)	36 (64.3)	609 (64.8)	69 (36.7)
TF	113 (50.2)	46 (62.2)	44 (16.2)	17 (24.1)	51 (11.5)	16 (28.6)	208 (22.1)	76 (40.4)
TI +/− TF	51 (22.7)	16 (21.6)	64 (23.6)	23 (39.7)	8 (1.8)	4 (7.1)	123 (13.1)	43 (22.9)
Swab positivity								
Ocular	152 (67.9) *	50 (67.6)	38 (14.0)	14 (24.1)	34 (7.8) *	9 (16.1)	224 (24.0)	73 (38.8)
Nasal	—	44 (59.5)	—	16 (27.6)	—	4 (7.1)	—	64 (34.0)

Data are the number (percentage of the total group).

* One individual from Maindi and seven from Jareng did not have ocular infection information.

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Table 2

Individuals with Amplicor-Positive Nasal Discharge at Baseline according to Clinical Trachoma Status and Ocular *C. trachomatis* Positivity

	Maindi	Rombo	Jareng	Overall*
Trachoma status				
Neither TF nor TI	2/12 (16.7)	1/21 (4.8)	0/36 (0.0)	3/69 (4.4)
TF	28/46 (60.9)	3/14 (21.4)	2/16 (12.5)	33/76 (43.4)
TI +/− TF	14/16 (87.5)	12/23 (52.2)	2/4 (50.0)	28/43 (65.1)
Total	44/74 (59.5)	16/58 (27.6)	4/56 (7.1)	64/188 (34.0)
Ocular swab positivity				
No	8/24 (33.3)	6/44 (13.6)	0/47 (0.0)	14/115 (12.2)
Yes	36/50 (72.0)	10/14 (71.4)	4/9 (44.4)	13/22 (59.1)
Total	42/74 (59.5)	16/58 (27.6)	4/56 (7.1)	64/188 (34.0)

Data are the number (percentage of the total group).

* $P < 0.0001$ Mantel-Haenszel test for trend, overall for clinical trachoma status, and ocular swab positivity.

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Table 3
Characteristics of Ocular Positivity at 2 Months among Treated Participants

Characteristic	Maindi*	Rombo	Jareng	All Sites	P†
Total participants	14/61 (23.0)	7/55 (12.8)	6/53 (11.3)	27/169 (16.0)	
Age (y)					
0.5 to <2	1/10 (10.0)	0/6 (0.0)	1/8 (12.5)	2/24 (8.3)	
2 to 3	5/23 (21.7)	4/16 (25.0)	3/24 (12.5)	12/63 (19.0)	0.56
4 to 5	5/17 (29.4)	2/19 (10.5)	2/13 (15.4)	9/49 (18.3)	
6 to 8	3/10 (30.0)	1/14 (7.1)	0/8 (0)	4/33 (12.1)	
Baseline nasal swab positivity					
No	2/24 (8.3)	1/39 (2.6)	4/49 (8.1)	7/112 (6.3)	
Yes	12/36 (33.3)	6/16 (37.5)	2/4 (50.0)	20/56 (35.7)	<0.0001
Baseline ocular swab positivity					
No	3/19 (15.8)	1/41 (2.4)	1/44 (2.2)	5/104 (4.8)	
Yes	11/41 (26.8)	6/14 (42.9)	5/9 (55.6)	22/64 (34.4)	<0.0001

Data are the number of subjects with positive ocular swabs at 2 months/total number of subjects (%).

*One participant did not have an ocular swab taken at 2 months, but was examined at 2 months. Both nasal and ocular swabs at baseline were negative.

†χ^2 *P*.

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Table 4

Adjusted Associations between Nasal Infection at Baseline and Ocular Infection at 2 Months among Participants Treated at Baseline

	OR	95% CI
Female	0.52	0.19–1.41
Study site		
Maindi	1.0	Reference
Rombo	1.26	0.39–4.08
Jareng	2.37	0.60–9.37
Baseline nasal swab positivity		
No	1.00	Reference
Yes	5.15	1.54–17.23
Baseline ocular swab positivity		
No	1.00	Reference
Yes	6.97	2.0–24.54

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Table 5

Individuals with Ocular Swab Positivity at 2 Months by Baseline Nasal and Ocular Swab Status

	Ocular Positivity at 2 Months		
Baseline Positivity Status	**No**	**Yes**	**% Infected**
No ocular, no nasal *	89	1	1.1
Ocular only	16	6	27.3
Nasal only	10	4	28.6
Ocular and nasal	26	16	38.1
Total	142	27	16.0

Data are the number and percentage of individuals infected.

* One participant missing ocular positivity status at 2 months.