Assessing the Prevalence of Trachoma: Clinical Examination or PCR?

Purpose

The purpose of this paper is to review the methods of assessing the prevalence of trachoma, particularly the potential role of polymerase chain reaction (PCR), and to address the frequent discordance between different methods.

This paper has been written to provide guidance to health practitioners and policy makers to assist them in decision making around how to assess the prevalence of trachoma.

It is important to note that the Communicable Disease Network of Australia took the position in 2013 that "Laboratory tests including nucleic acid amplification tests (e.g. polymerase chain reaction or PCR) are available, but the results do not correlate well with clinical signs and are not recommended for routine use."[1]

Introduction

A simple reliable assessment method is needed to establish the prevalence of trachoma and determine whether or not trachoma has been eliminated as a public health problem, and to undertake ongoing surveillance for possible recrudescence. The clinical diagnosis of trachoma is fairly straight forward to make, although some training and confidence is required.[2, 3] Over the years, a number of different methods have been explored to try to get a reliable laboratory method to diagnose trachoma.[4]

History of clinical grading for trachoma

The MacCallan Classification of trachoma was first introduced in 1908 and became the foundation for trachoma grading for decades.[5] It was a modification of the various descriptions of the stages of trachoma that date back to the 5th Century AD.[6] Starting in 1952, the World Health Organisation (WHO) Expert Committees came up with increasingly complicated derivations of the MacCallan Classification.[7] In 1966, the WHO Expert Committee came up with some 20 signs of trachoma to be graded by slit lamp examination, and severity ratings ranging from two to five were assigned.[8] After a further revision, a new WHO grading system was published in 1981 that had five signs each graded zero to four.[9] Again, this approach required slit lamp examinations.

The experience of using simplified grading criteria during the Australian National Trachoma and Eye Health Programme, and in field studies in Malawi and Mexico, led to the development of a new WHO Simplified Grading Scheme in 1987.[2,4] The Simplified Grading Scheme aimed to provide a practical way for community-level health workers and others to assess prevalence of blinding trachoma as a public health problem with some degree of certainty.[10] The threshold for each sign of trachoma was set at a fairly high level so that there was certainty of the presence of the particular sign. It was anticipated that the rates found using this method would reflect the public health significance of trachoma. The

Simplified Grading Scheme was not designed to be used to assess and diagnose individual cases of trachoma, nor was it designed to replace the 1981 WHO Grading for detailed scientific studies, especially those assessing various laboratory-based tests of diagnosis. Rather, it was designed as a public health tool for measuring community-level prevalence of trachoma.

To give a higher level of reproducibility, the Simplified Grading should be done with x2.5 magnifying loupes or glasses. This is particularly important when trachoma grading is being undertaken during a prevalence survey. Ideally, for occasional screening in a clinical setting x2.5 loupes should also be used. However, if they are not available, varying degrees of magnification could be used ranging from none - relying on a naked eye examination - to the use of a slit lamp.

The history of laboratory grading of trachoma

Work started on the laboratory diagnosis of trachoma when it became possible to culture chlamydia in egg yolks in 1957.[11] Although Giemsa stain has been in use for individual diagnosis since 1907,[12] it was not routinely used for large scale screening programs.

Culture in eggs or later in tissue culture proved to be too time consuming, difficult, and expensive to offer a practical diagnostic tool, but culture did provide large quantities of chlamydia that could be used to develop serologic tests.[11]

In the 1970s, a lot of work was done by different groups using a variety of serologic diagnostic tools.[13] The most widely used was the Micro-immunofluorescence assay, Micro-IF, developed in Seattle.[14] Micro-IF could be used on conjunctival scrapings and, later in 1982, when monoclonal antibodies became available these Direct Immunofluorescence Assays were found to be far superior to Giemsa stain.[15]

Extensive field studies in the early 1970s examined the role of antibody detection as another method of diagnosing trachoma.[16,17] Antibodies to chlamydia can be found in serum and tears, they include IgM, IgG and IgA. Some studies used serotype specific antibodies in an attempt to improve sensitivity and specificity. However, although tear anti-chlamydial IgG and IgA correlated with the presence of active trachoma, the correlation was not strong and was less specific than identifying the chlamydia in smears using Micro-IF.

More recent studies assessing the role of serology have used antibodies to specific chlamydial antigens.[18] These have shown low prevalences of seropositivity across age groups without a clear linkage to the presence or absence of TF,[10] or “active trachoma”. However, most of these studies have not used the more detailed methods of grading the presence of trachoma such as the 1981 WHO grading system. In summary, it is unclear if serology will be useful in monitoring trachoma prevalences or its recrudescence, although future tests of chlamydia-specific tear IgA antibody levels may have some role.

Polymerase Chain Reaction (PCR)

History of PCR and its use in trachoma detection

Polymerase Chain Reaction was first used in 1989 as probes to detect chlamydia in trachoma.[19] These tests had a high sensitivity and were as specific as culture. Since then, many studies of trachoma have been done using PCR and over time, the commercially available tests have improved considerably and are now seen as routine.

Challenges and risks of using PCR to detect trachoma

PCR has some specific issues, one of which is its high sensitivity and the ease with which specimens can be contaminated unless careful no-touch procedures are used to collect the samples. The person collecting the sample and anyone else assisting, such as by holding the head or flipping the eyelid, must have clean sterile gloves. The assays detect nucleic acids, either DNA or mRNA. However, nucleic acids can persist in dead organisms for some time and so a positive test does not necessarily mean that the test has identified a viable or infectious organism.

Collecting PCR swabs properly is not a minor undertaking. To obtain a reliable swab, one must evert the upper eyelid and use the collection swab to vigorously rub the conjunctival surface. A swab of the tears in the lower fornix is inadequate. At times, local anaesthetic may be needed as the swabbing of the everted lid is uncomfortable and many children do not like it. If local anaesthetic drops have been used, care must be taken to make sure that people, particularly children, do not rub their eye after swabbing because of the risk of a corneal abrasion. Further, the load of PCR positive organisms can decrease by more than one log unit between the first swab and a second repeat swab.[20]

Specimens need to be properly identified, stored carefully, shipped securely, and results returned in a timely fashion to be useful - and this has significant cost and logistical implications. The costs of a single test range from about $10 to $20. If the test were done in a private pathology laboratory, currently it would be billed at $36.60.

Correlation between clinical disease and the presence of infection

Trachoma is a very unusual infection. A single episode of chlamydial eye infection will resolve spontaneously over a month or two and leave no long-term sequelae or scarring. This is known as Inclusion Conjunctivitis. Organisms can be identified by most laboratory tests for the first few weeks or so.

The “chronic” blinding disease trachoma needs multiple episodes of reinfection to sustain the inflammatory response. Each episode of infection stimulates and maintains the ongoing inflammatory response that with time leads to scarring in the eyelid, in-turning of the eyelashes, and consequently, blindness. However, despite the need for ongoing reinfection and persistent inflammation, the organism is not always detectable. One estimate is that 150 episodes of infection are needed to cause the late stage of trachoma, trichiasis[21] and animal models suggest re-infection is required every week to maintain ongoing trachoma.[22] In this animal model with weekly re-inoculation, active trachoma continued even though after a few months the presence of chlamydia was only occasionally found. However, if the

weekly re-inoculation was stopped, the disease resolved over a few months[23]. In humans, in the absence of continuing transmission and reinfection, well-established active trachoma, TF, will usually resolve in three to nine months. Repeated inoculation is essential to maintain the active disease even though the organism is only rarely identified.

Even with the most sensitive test, PCR will typically only confirm one quarter of those children with well-established active trachoma, TF[24] and only two thirds or so of those with the more intense inflammation, TI.[10] PCR is therefore not a useful or reliable measurement tool for the prevalence of trachoma.

A study in the Katherine region of the Northern Territory found that only 18 percent of those with TF were PCR positive (Figure 1).[20] Further, those with TF formed only 41 per cent of those who were PCR positive. This study used a fine clinical grading to assess trachoma and found that 46 per cent of those who were PCR positive had active trachoma but it was not severe enough to be graded as TF. There were also a few people who were PCR positive who did not have follicles, most of them were older women often with inflammation and scarring who lived in houses with others who had TF, some of whom were also PCR positive.

Those with more severe clinical disease were also more likely to be PCR positive and to have a higher infectious load. Similar findings are seen as the prevalence to trachoma increases in hyperendemic areas.[24] Equally, for those with less severe disease or from areas with lower prevalences of trachoma, the correlation becomes even less strong. Further, after the distribution of antibiotic treatment, the levels of infection drop very quickly while the clinical response is much slower. Unless transmission is significantly reduced by improved hygiene, levels of both PCR positivity and clinical disease will slowly return to pre-treatment levels.

Why do we have active trachoma and yet chlamydia can only be demonstrated in the minority of cases?

The answer lies in the pathogenic mechanisms that underlie trachoma. Much of this understanding has come from animal studies where the conditions and episodes of infection can be controlled and specimens or biopsies collected when required.[4]

Studies in several species show that it is the immunologic response to chlamydial infection that leads to the tissue changes and damage we see in trachoma.[25,26] Chlamydia replicate inside the conjunctival epithelial cells. In the laboratory, this takes some four to eight days and it is probably similar in vivo. During replication, an antigenic protein, identified as HSP60, is released and this stimulates a Type 4 or Delayed-Type Hypersensitivity reaction, DTH. These DTH reactions are a cell mediated response to an antigen that involve immune T-cells interacting with inflammatory monocytes and macrophages.[22,27] These cells overproduce cytokines, leading to inflammation with germinal centres (follicles) and fibroblast proliferation leading to scarring.[28,29] With time and ongoing inflammation, the scarring contracts and distorts the upper eyelid causing the lashes to turn in and rub on the eye –known as trichiasis. The lashes rub the cornea and so lead to corneal scarring and blindness.

Allergic contact dermatitis is a commonly recognised DTH reaction.[30] This may occur to metals such as nickel or chemicals such as those found in poison ivy or poison oak. In poison ivy, for example, even a brief touch with a leaf by someone who has been sensitised will lead to a severe skin reaction that can last for weeks, even if the antigen is washed off the skin straight away.

In trachoma, brief episodes of exposure and infection lead to prolonged inflammation. The longer the inflammation continues, and the more severe it is, the more the damage and scarring occurrs, and the greater later risk of trichiasis and blindness.

Screening for trachoma: What does all of this mean and where does it lead us?

Clinical examination
The advantages of clinical examination are that:

- it is quick and the results are immediately available
- there are no added costs
- it assesses the disease in question
- while some are hesitant to evert or 'flip' the eyelids of children, this is a simple clinical skill that is easy to learn
- for prevalence surveys x2.5 magnifying loupes should be used, but in a clinical setting, if magnification is not available, a naked eye exam is acceptable.

Serology testing:
The disadvantages of serology testing are that it:

- has not shown any real promise, despite being around for some 60 years
- requires the collection of blood or tears
- incurs significant costs(disposables, storage, transport, and laboratory costs).

PCR testing
The disadvantages of PCR are that:

- it still involves flipping the eyelid
- it is complex and uncomfortable
- it may require the use of topical anaesthetic
- It incurs significant costs(disposables, storage, transport, and laboratory costs).
- it grossly underestimates the prevalence of active trachoma, TF, and yet detects many who may have clinical disease but not severe enough to be graded as TF
- the Communicable Diseases Network of Australia recommends against using it for the screening of trachoma.

Conclusion

Given these issues – at least for the foreseeable future – the Communicable Diseases Network of Australia recommendation for clinical grading as the most reliable and appropriate method for assessing the prevalence of trachoma stands; it is rapid, acceptable to subjects, and highly cost-effective.

	n	PCR-positive (*%*)	
Population	1282	46	(3.6)
Simplified grading			
- TF Absent	1,174	27	(2.3)
- TF Present	108	19	(17.6)
Fine grading			
- TF_0 - normal	793	6	(0.8)
- TF_1 - minimal	257	16	(6.2)
- TF_2 - mild	124	5	(4.0)
- **TF_3 = TF**	**98**	16	(16.3)
- **TF_4** -severe	**10**	3	(30)

Fig. 1 Correlation between clinical signs of trachoma and PCR positioning in Katherine region[19]

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