

munity child health clinics is feasible, and furthermore that routine blood sampling is acceptable to parents, so answering the first question posed in *Health for all Children*.⁴ The second question put by this document was whether the incidence of iron deficiency could be reduced by appropriate health education. This remains to be answered. The third question was 'what measurable benefits may result from a more aggressive approach to the identification and treatment of iron deficiency?' Although problems associated with iron deficiency have been identified and shown to respond to treatment with iron, the natural history of iron deficiency anaemia in childhood is not understood. It may be that deficiency resolves with age, or recurs after a period of treatment.

Considering the incidence, importance, and reversibility of iron deficiency anaemia it seems clear that intervention is necessary. Estimation of haemoglobin concentration by fingerprick is feasible in the community, and

acceptable to parents. Evaluation is not yet complete enough to formulate a nationwide policy. The results of our study, however, justify special consideration for high risk subgroups in the meantime.

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Diagnosis of neonatal chlamydial conjunctivitis

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Abstract

Fifty seven babies with ophthalmia neonatorum had conjunctival smears examined by microscopy and bacterial culture, and by immunofluorescence, to find out which was the best method of diagnosing chlamydial conjunctivitis. The positive (33%) and negative (70%) predictive values of microscopy and culture were too low for us to accept it as an adequate method of detecting the presence of *Chlamydia trachomatis*.

Chlamydia trachomatis is a common cause of generally benign ophthalmia neonatorum.¹ It may also cause pneumonia.² The mother's genital tract is infected and she may develop ascending infection, pelvic inflammatory disease, and (perhaps) infertility. The costs of chlamydial infections in the United States have been estimated at more than \$1.4 billion a year; much of this is for the treatment of pelvic inflammatory disease.³ Sexual contacts of the mother, if untreated, may spread infection.

Wincelous and colleagues diagnosed chlamydial ophthalmia neonatorum using simple laboratory techniques, which are valuable in laboratories lacking diagnostic facilities for chlamydial infection.⁴ We have evaluated similar simple techniques using immunofluorescence for comparison (MicroTrak *Chlamydia trachomatis* direct fluorescent antibody reagent, Syva UK).

Patients and methods

Fifty seven babies between 1 and 42 days old in

hospital with acute conjunctivitis were studied; 51 were less than 21 days old. None had received topical antibiotics.

After removing any exudate, two swabs were firmly taken from the inflamed tarsal conjunctivas. One swab was rolled on to the unmasked area of a microscope slide (Syva UK), allowed to dry, then fixed with acetone.

Another ordinary smear was made from this swab. The second swab was sent in Stuart's transport medium for bacterial culture. Specimens were sent as soon as possible. Masked slides were stained, either on arrival in the laboratory or after overnight storage at 4°C (or at -20°C if longer storage was required).

LABORATORY METHODS

Staining of slides by immunofluorescence

Firstly, 25 µl of MicroTrak direct specimen stain reagent (*Chlamydia trachomatis*-direct fluorescent antibody reagent) were added to the unmasked area of the slide. This was incubated at room temperature for 15 minutes, then rinsed, and mounted with MicroTrak mounting fluid.

The preparation was examined for chlamydial particles by incident light fluorescence and a ×50 water immersion fluorescence objective.

Giemsa staining of slides

Slides were fixed with methyl alcohol, stained with Giemsa stain, and examined by light microscopy using a ×54 oil immersion or ×100 oil immersion objective, for the presence of any polymorphonuclear leucocytes and bacteria.

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Results of microscopy and detection of chlamydial antigen

	Polymorphonuclear leucocytes present; bacterial pathogens not present	Polymorphonuclear leucocytes not present; bacterial pathogens not present	Total
Chlamydial antigen detected	9 (8)	8 (6)	17 (14)
Chlamydial antigen not detected	18 (7)	19 (8)	37 (15)
Total	27 (15)	27 (14)	54 (29)

Numbers in parentheses refer to 'bacterial pathogens and commensals not present'.

Microscopy

The results of one observer's findings were not known by the other, who read the corresponding slide stained by the alternative method.

Culture of conjunctival swabs

Conjunctival swabs were inoculated on to blood and chocolate agar plates for aerobic incubation with 5% carbon dioxide, and on to blood agar for anaerobic incubation with 5% carbon dioxide, both at 37°C overnight. Organisms were identified to species level.

Results

INTERPRETATION OF FINDINGS ON IMMUNOFLUORESCENCE

When 10 or more typical elementary or reticulate bodies were seen on the whole slide the result was considered positive, and if there were less than 10 such particles it was considered negative. Most positive slides contained more than 20 particles; there were three slides recorded as negative with six, four, and three particles, respectively.

MICROSCOPY AND CULTURE

The results are in the table. Pathogens referred to were *Staphylococcus aureus*, streptococci (Lancefield groups A, B, C or G), *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas spp.*, and *Neisseria gonorrhoeae* and meningitidis; the sensitivity and specificity of the presence of polymorphonuclear leucocytes in the absence of such pathogens as indicative of chlamydial infection were 53% and 51%, and the positive and negative predictive values, 33% and 70%, respectively.

Scanty colonies of *Staphylococcus epidermidis*, *Streptococci viridans*, other species of neisseria, and coliforms were considered as commensals; the sensitivity, specificity, and positive and negative predictive values when polymorphonuclear leucocytes were present and bacteria (including commensals) were absent, were 57%, 53%, 53% and 57%.

Discussion

The incidence here of conjunctival chlamydial infection was 17 of 57 (30%), in keeping with the findings of others.⁵

Winceslaus *et al* showed that the presence of polymorphonuclear leucocytes without bacteria indicated that the infection was probably chlamydial.⁴

In our study, however, sensitivity and specificity of this method were low, and positive and negative predictive values were unacceptable, whether bacterial pathogens alone, or any bacteria, were considered.

If these criteria had been applied, 18 babies with no pathogenic bacteria present, and seven with neither pathogenic bacteria nor commensals present,—all of whom showed no antigen on immunofluorescence—would have been falsely considered to have chlamydial infection.

Our results, though obtained differently, do not suggest that the presence of polymorphonuclear leucocytes without bacteria necessarily indicate neonatal chlamydial conjunctivitis, nor does the converse apply.

We accept that immunofluorescence is inferior to cell culture for the detection of *C trachomatis*. Moreover, we accepted 10 or more characteristic particles on each slide as positive; this is higher than some have accepted as positive and depresses sensitivity and positive predictive value further.⁶ Our inability to detect chlamydial antigen in 18 instances when polymorphonuclear leucocytes were present but no bacteria, and seven when they were present with neither bacteria nor commensals, could have been explained in part by these factors. It is unlikely, however, that even taken together these would have produced such a high proportion of false negative results.

There were also eight (six with neither commensals nor pathogens) cases positive on immunofluorescence in which neither polymorphonuclear leucocytes nor bacteria were detected; there would thus have been eight (six) false negative results. It is unlikely that in removing exudate before taking conjunctival swabs for microscopy all polymorphonuclear leucocytes would have been removed.

We are still unable to explain why our results differ from those of Winceslaus *et al*,⁴ and further studies are needed to clarify this. In the meantime, however, we think that it would be premature to base prediction of chlamydial infection on the presence of polymorphonuclear leucocytes and the absence of bacteria in conjunctival specimens.

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