Detection of *Chlamydia trachomatis* in general practice urine samples

M S DRYDEN

M WILKINSON

M REDMAN

M R MILLAR

SUMMARY

Background. Chlamydia trachomatis *is frequently overlooked as a cause of dysuria and urinary frequency in general practice patients.*

Aim. *This study set out to determine the impact of performing chlamydial antigen detection on sterile pyuria samples from patients aged 16–65 years and which were submitted to a hospital microbiology laboratory by general practitioners in the Winchester health district for routine microbiological investigations.*

Method. *Chlamydial antigen detection was performed by enzyme immunoassay and direct immunofluorescence. The cost of performing the test was estimated. In the first year of the study (1991) questionnaires were sent to general practitioners whose patients had a positive test result.*

Results. *A total of 1025 samples of sterile pyuria were received at the laboratory between January 1991 and March 1993. Chlamydial antigen was detected in 54 samples (5%); 22 men and 32 women aged between 16 and 57 years (mean 25 years). The detection rate was highest in the 16–20 years age group (22% of men had a positive sample and 7% of women). Completed questionnaires from 27 general practitioners revealed that 59% of their patients were referred to the genitourinary clinic for treatment and contact tracing. The others were treated by the general practitioner. The cost of the screening programme per cure in this population was estimated to be £246.*

Conclusion. C trachomatis *is a significant pathogen which may go unrecognized and untreated. The cost, medically and financially, of screening for this pathogen and treating infected patients and contacts is likely to be less than ignoring it, particularly if screening is confined to the 16–30 years age group. General practitioners should consider the diagnosis of chlamydial infection in young adult patients with sterile pyuria, and microbiology laboratories should screen sterile pyuria samples for chlamydial antigen.*

Keywords: Chlamydia trachomatis; *urinary tract infections; urination disorders; urine testing.*

Introduction

THE urogenital pathogen, *Chlamydia trachomatis*, may cause dysuria and frequency of urination,[1] and such symptoms are commonly encountered by general practitioners. However, *C trachomatis* is often unsuspected as a cause of dysuria, particularly in women, and may be difficult to diagnose. The investigation of dysuria by general practitioners usually involves the collection of a mid-stream specimen of urine, and the routine techniques employed by the majority of microbiology laboratories to identify pathogens in urine samples will not detect the presence of *C trachomatis*. In addition empirical antibiotic treatment for urinary tract infection, commonly a beta-lactam antibiotic, trimethoprim, or nitrofurantoin is unlikely to eradicate chlamydia. The consequences of inadequate treatment are considerable. The infection may be transmitted to sexual partners, and complications in both the index case and contacts include infertility, pelvic inflammatory disease, epididymo-orchitis, prostatitis and neonatal infection.[2]

C trachomatis may be detected in urine samples by established non-culture methods including enzyme linked immunoabsorbent assay and direct immunofluorescence.[3] Most microbiology laboratories now offer these two methods of detection. Detection of chlamydial antigen by enzyme immunoassay in urine is an effective method of screening symptomatic[4] and asymptomatic patients[5] and may be useful for epidemiological purposes.

The benefits of identifying cases of urogenital chlamydia infection early are clear. Patients can be provided with an early diagnosis of their condition and managed appropriately. This may result in a reduction in the incidence of complications and, with contact tracing, permit treatment of secondary cases. It is likely that there would be a cost benefit in identifying and treating unsuspected cases of urogenital chlamydia infection, to the general practice in reducing recurrent visits, to the health service in the reduction of complications of the infection, and to the community in the reduction of sexually transmitted disease. Such advantages need to be weighed against the possible disadvantages of chlamydia testing: the cost of performing the test, the evaluation of the test and the possibility of false results, and the difficulties to the patient and general practitioner of unexpected results, particularly when the test has not been requested.

The purpose of this study was to determine the value and cost of testing sterile pyuria samples from general practice in order to identify cases of urogenital chlamydia infection which may otherwise have gone unrecognized. In order to assess the impact of the results, questionnaires were sent to the general practitioner of each infected patient in the first year of the study.

Method

Patients, samples and laboratory procedures

Between January 1991 and March 1993 all mid-stream specimens of urine received by the microbiology laboratory of the Royal Hampshire County Hospital from general practitioners for routine urinalysis and culture were considered for inclusion in the study. Urine samples from which bacteria were isolated, or in which the request form indicated recent antibiotic therapy, were excluded. All other sterile pyuria samples from men and women patients aged between 16 and 65 years were included, unless the presence of antimicrobial agents was detected. Sterile pyuria was defined as the presence of >10 white blood cells per high power field on microscopy and with no bacterial growth following 18

M S Dryden, MA, MSc, MD, MRCPath, consultant microbiologist; M Wilkinson, AIMLS; and M Redman, FIMLS, medical laboratory scientific officers, Department of Microbiology, Royal Hampshire County Hospital, Winchester. M R Millar, MD, MRCPath, senior lecturer, Department of Microbiology, Bristol Royal Infirmary.

Submitted: 11 January 1993; accepted: 28 July 1993.

hours' incubation of 2 µl urine on cystine lactose electrolyte deficient agar (Oxoid CM301®, Basingstoke). Samples were kept at 4 °C and processed daily.

In the first part of the study, to the end of March 1991, all samples included were tested for the presence of antimicrobial agents. Diagnostic sensitivity test agar (Oxoid CM261®) was seeded with a suspension of a sensitive strain of *Escherichia coli* in a concentration sufficient to give a confluent growth after incubation. Eight 5 mm filter paper discs were applied to each agar plate, and to each disc 10 µl urine was added. A zone of growth inhibition of >3 mm around the disc after incubation was regarded as evidence of the presence of antimicrobial substances. In addition, some samples were tested for the presence of nutritionally dependent bacteria by culturing 0.2 ml urine on chocolate agar at 37 °C in 7% CO_2; these were excluded if positive.

Enzyme immunoassay (Microtrak®, Syva) was used to screen all pyuria samples for the presence of chlamydial antigen. Samples found to be positive by enzyme immunoassay were confirmed by direct immunofluorescence (*C trachomatis* direct specimen kit®, Syva). Both screening techniques were carried out according to the manufacturer's instructions. The investigations were carried out on the deposit of 5 ml of urine centrifuged at 3000 rpm after resuspension in 1 ml of the transport medium supplied with each kit.

Samples found to be positive by enzyme immunoassay but negative by direct immunofluorescence were regarded as false positives. A result was regarded as a true positive if both tests were positive and a report of a positive result was sent to the general practitioner, together with a comment suggesting referral of the patient to a genitourinary clinic for treatment and contact tracing. General practitioners were also told of patients' negative chlamydia results.

Questionnaire

Positive reports were followed three weeks later by a questionnaire to the general practitioner during the first half of the study from January 1991 to February 1992. Questions included the effect of the result on the management of the patient, whether specialist referral and contact tracing had been sought, and whether the result was of benefit to the patient.

Costings

The cost of performing chlamydial detection, £7.84, was determined by a combination of staff costs, capital costs and overhead expenses and the cost of consumables. In calculating the cost of treatment it was assumed that infected patients required a further general practitioner consultation and follow up, or referral to a genitourinary clinic. The cost of these was estimated at £25.00 per patient. The cost of seven days' treatment with doxycycline 100 mg 12 hourly was £8.00. Regarding contact tracing, an assumption was made that for every infected patient, 1.5 contacts needed to be traced. The cost of tracing was determined as follows: administration £2.00, two clinical consultations totalling £25.00, chlamydial testing £7.84, treatment £8.00, giving a total cost per contact of £42.84, or a cost of contact tracing for each index case of £64.26. The cost per cure was the sum of the total costs of the screening programme plus treatment costs plus contact tracing costs, divided by the number of infected patients. The cost of missing a diagnosis of chlamydia could not be determined because there were too many variables in terms of the risk of transmission and development of complications.

Results

Detection of chlamydia

In the 27 month period of the study 66 510 urine samples were processed by the laboratory; 38 025 (57.2%) were received from general practitioners and of these, 1025 (2.7%) were samples of sterile pyuria. Sterile pyuria samples were most commonly received from women patients aged between 16 and 40 years (614 samples, 59.9%). Only one of 112 tested samples was found to contain antimicrobial agents, and one of 270 samples yielded nutritionally dependent microbes; these were therefore excluded.

Chlamydia antigen was detected in 54 (5.3%) of the urine samples tested by both enzyme immunoassay and direct immunofluorescence, and these were regarded as true positives. Twelve samples were found to be positive by enzyme immunoassay but negative by direct immunofluorescence, and were regarded as false positives. The 54 patients with chlamydial infection (22 men and 32 women) were aged between 16 and 57 years, mean age 25.4 years. Their sex and age distribution is shown in Table 1. The proportion of chlamydia positive samples by age was highest in the 16 to 30 years age groups, especially among men.

Costs

The cost of performing a chlamydia test was £7.84 per sample, giving a total cost for testing the 1023 samples of sterile pyuria of £8020.32. The cost of treating the 54 detected patients was £1782.00 and that of appropriate contact tracing was £3470.04, giving a grand total of £13 272.36. Therefore the cost per cure was £245.78.

Questionnaires

Of 36 questionnaires sent to general practitioners during the first half of the study 27 (75%) were returned. Four of the 27 general practitioners had made an initial diagnosis of urethritis, in 20 the initial diagnosis was a urinary tract infection, in one antepartum haemorrhage and in one postpartum abdominal pain (no clinical diagnosis was given in one case). Nine doctors had also considered the possibility of a diagnosis of a sexually transmitted disease before receiving the result.

After receipt of the result 10 patients received treatment from their general practitioners without specialist referral: four were prescribed tetracycline, two erythromycin and four unknown, and contact tracing was carried out in five of these cases. One further patient refused to accept the diagnosis, but did accept appropriate treatment, and showed a therapeutic response. Sixteen patients were referred to the genitourinary clinic for treatment and contact tracing.

The general practitioners were asked how the test result had contributed towards the management of the patient. Twenty replied that the result was beneficial to the patient for the following reasons: because it permitted accurate diagnosis (10), appropriate treatment (seven) and specialist referral (three). Six general practitioners felt that the result was detrimental to the patient for the following reasons: because it caused worry and anxiety

Table 1. Sex and age distribution of the 54 patients with a positive chlamydia test result.

	No. of positive samples (% positive of sterile samples in group)	
	Men	Women
Age (years)		
16–20	8 (*21.6*)	14 (*7.2*)
21–30	8 (*16.7*)	13 (*5.0*)
31–40	3 (*6.5*)	3 (*2.2*)
41-50	1 (*2.0*)	2 (*2.0*)
51-60	2 (*5.1*)	0

(two), it affected the relationship with the partner (one), the test had not been requested (one), and there was difficulty maintaining confidentiality in view of the patient's young age and parental involvement (two). One doctor gave no answer.

Discussion

Chlamydia trachomatis is a common cause of non-specific urethritis, cervicitis and the urethral syndrome.[1] Patients with any of these conditions may present with dysuria and urinary frequency as their predominant symptoms, and yet chlamydia is frequently overlooked as a cause and rarely sought in routine urine microbiological investigations. The consequences of failure to eradicate this infection may be severe and include pelvic inflammatory disease, infertility, epididymitis and chronic prostatitis, and neonatal infection.[2]

This study has demonstrated that routine mid-stream specimens of urine may be used as samples to detect chlamydia in patients with sterile pyuria, avoiding the necessity to collect cervical or urethral swabs, and providing a diagnosis which in many cases had not been considered. A wide age range of patients was studied, with a detection rate of 5%. However, the rate was strikingly high in young adults: 22% men and 7% of women aged between 16 and 20 years with sterile pyuria had chlamydial infection.

Other studies have shown that approximately 8% of women with the urethral syndrome, who often have sterile pyuria, are infected with *C trachomatis*.[1] Using laboratory techniques similar to this study to test urine samples, the incidence of chlamydial infection was found to be 12% in asymptomatic Austrian soldiers,[6] and at a Swedish genitourinary clinic chlamydia was detected in the urine of 11% of men and 12% of women.[7] In sexually active American adolescent males with pyuria, urine and urethral swabs revealed *C trachomatis, Neisseria gonorrhoeae,* or both in 86%.[8] *C trachomatis* is a widespread and significant low grade pathogen in sexually active adults.

The detection of chlamydial antigen is usually performed on swabs of the urethra or cervix. Chlamydial enzyme immunoassay of urine has been used in patients at high risk of sexually transmitted disease, and also in groups of asymptomatic men.[7,8] Because chlamydia infection may be at the cervix rather than the urethra, enzyme immunoassay of urine may be less sensitive than detection of chlamydia from a cervical swab in women,[9,10] which may explain the lower detection rate in women in this study. Testing of urine samples in men has been shown to be as sensitive as testing urethral swabs.[7] Although testing urine samples may give a greater number of false negative results than testing urethral or cervical swabs, urine is a convenient sample to collect. The recommended sample of urine for chlamydial detection is the first 5 ml of urine passed. This study examined mid-stream specimens of urine which may have further reduced the sensitivity. However, there are clear advantages in favour of a mid-stream specimen of urine as an initial investigation in that it can be routinely cultured in addition to being processed for chlamydia detection.

There have been reports of false positive chlamydial results from urine contaminated with bacteria[11,12] but, although a few enzyme immunoassay false positives were observed in the present study, the reason was not bacteria contamination as the samples were all culture negative. If an enzyme immunoassay method is used to screen urine samples it is essential to confirm all positive results by direct immunofluorescence, particularly if patients are to be informed that they have acquired, and need to be treated for, a sexually transmitted disease.

Ninety five per cent of the sterile pyuria samples had a negative chlamydia test result. Antibacterial substances were only detected in one sample of those tested; this low figure may be explained by the fact that samples whose request form stated the use of antibiotics were not included in the study. The percentage of urine specimens containing antibacterial substances is usually between 16% and 20%.[13] A further small percentage of sterile pyuria is caused by infection with fastidious organisms which fail to grow on conventional media, although this was only detected in a single sample of those tested in this study. The aetiology of the sterile pyuria in the other cases is unknown. Possible causes of sterile pyuria include chlamydia not detected by the techniques used, *Mycoplasma* or *Ureaplasma* species, *Trichomonas vaginalis, Gardnerella vaginalis, N gonorrhoeae,* viral infection or tuberculosis, or inflammation of a non-infective aetiology.

The general practitioners' questionnaires raise a number of issues relating to routine chlamydial testing of urine samples. Although a third of the respondents had considered the possibility of sexually transmitted disease, conventional investigations to detect chlamydial infection before the positive urine test result was reported had not been performed, and in no case had the patient received appropriate anti-chlamydial therapy before the unsolicited report. Consequently, these infections might have gone undiagnosed and untreated, with progression to complications in some cases and uncontrolled transmission. As a result of the positive test, the majority of respondents' patients were referred to the genitourinary clinic, while some general practitioners treated their patients themselves. Most general practitioners regarded the unsolicited testing as beneficial to the patient, because it provided a diagnosis, permitted appropriate therapy or specialist referral. The negative replies generally related to the social and emotional difficulties for patients with a sexually transmitted disease.

One general practitioner clearly felt that the microbiology laboratory should not have performed a test which had not been requested. In practice, this occurs all the time in every laboratory, for example when a series of viral serology tests are performed in response to a non-specific request for viral titres. A direct parallel with urine chlamydia testing is the culture of all genital specimens for *N gonorrhoeae*. A specific request for the identification of this organism is rarely received for genital specimens collected outside the genitourinary clinic, but it is always performed and reported.

Screening the population of this health district for chlamydial infection, by examining sterile pyuria from patients between the ages of 16 and 65 years is cost effective. The cost of identifying and treating one infected patient by this method was calculated to be £246. This is likely to be considerably less than the cost of providing health care for individual patients and their contacts with complications of untreated chlamydial infection. The costing in this study has been crude and it is impossible to determine with any accuracy the cost of not screening. More accurate cost analysis would be a major undertaking and it is unlikely that the conclusions would be different. In an American cost analysis study of chlamydial screening in adolescent men the cost per infected man was estimated at $144 and the cost per case for infected women partners and transmission to neonates was calculated to be $365.[14] In the United States of America over four million chlamydial infections each year cost an estimated $1.5 billion. Cost analysis of this sort has not been performed in the United Kingdom. Some health districts in the UK may have a higher incidence of chlamydial infection than in Winchester, making such a screening programme more cost effective.

Screening sterile pyuria samples from general practice for chlamydia is likely to detect unrecognized and unexpected urogenital chlamydial infection. In conclusion, microbiology laboratories should consider routine chlamydia testing of sterile pyuria, particularly in young adults. Sterile pyuria should be tested by an

established enzyme immunoassay method and if this is positive, the result should be confirmed by another method such as direct immunofluorescence. If this is performed routinely, it is likely to be medically and financially cost effective. It should be recognized that this method of screening is probably more sensitive in men than women. General practitioners should consider the diagnosis of chlamydial infection in patients with sterile pyuria, and in women if the urine test result is negative appropriate cervical swabs should be collected. In the long term, with adequate contact tracing and treatment, such a screening programme may be effective in reducing the incidence of chlamydial infection.

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Address for correspondence

Dr M S Dryden, Department of Microbiology, Royal Hampshire County Hospital, Winchester, Hampshire SO22 5DG.