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Tubal damage in infertile women: prediction using chlamydia serology

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BACKGROUND: The study explores the relationship between serum chlamydia antibody titres (CATs) and detection of tubal damage in infertile women. METHODS: The tubal status and pelvic findings in 1006 women undergoing laparoscopy for infertility were related to CAT, which was measured using the whole-cell inclusion immunofluorescence test. RESULTS: A negative correlation between CAT and age was noted. A linear trend between serum CAT and the likelihood of tubal damage, including severe damage, was observed ($P < 0.001$). Titres in women with tubal damage (median 1:1024; range <1:64–1:4096) were significantly ($P < 0.001$) higher than in women with endometriosis alone (median <1:64; range <1:64–1:512) or those with a normal pelvis (median <1:64; range <1:64–1:1024). Women with positive titres were more likely to have pelvic adhesions than tubal occlusion unless titres were very high, when tubal damage was likely to be more severe. CONCLUSIONS: CATs are of predictive value in the detection of tubal damage and are quantitatively related to the severity of damage. For practical clinical purposes, Chlamydia serology is useful mainly as a screening test for the likelihood of tubal damage in infertile women and may facilitate decisions on which women should proceed with further investigations without delay.

Key words: chlamydia/infertility/pelvis/serology/tubal damage

Introduction

Couples may present to their family doctor complaining of infertility after failing to conceive for months or years. Those with a short duration of infertility will mainly be normal and have simply been unlucky so far (Hull, 1992). In primary or secondary care of infertile couples, the principal aim is to screen for a likely cause deserving early referral for specialist diagnosis and treatment. Other infertile couples can reasonably be managed by simple advice and encouragement before specialist help can be justified. The clinical challenge is deciding which couples deserve early referral to a specialist for further investigation or treatment.

Tubal damage is a common cause of infertility, and laparoscopy or hysterosalpingography (HSG) are accepted methods for diagnosing this condition. They are, however, both costly and invasive, and therefore unsuitable for screening on a large scale. Genital *Chlamydia trachomatis* infection has a worldwide distribution (Stamm, 1999) and is now recognized as the single most common cause of tubal peritoneal damage (WHO task force on the prevention and management of infertility, 1995; ESHRE Capri Workshop, 1996). Infection with *C.trachomatis* results in the formation of antibodies detectable in serum. In contrast to laparoscopy or HSG, detecting evidence of past chlamydial infection using serology is
non-invasive, simple and quick to perform (Akande, 2002). As such, chlamydia serology may be used as a screening test for tubal damage in infertile women. This study explores the relationship between chlamydia antibody titres (CATs) and tubal damage in infertile women undergoing investigative laparoscopy.

Materials and methods

Local research ethics committee approval was obtained for the study. The study group was drawn from infertile women whose initial attendance at the Reproductive Medicine Clinic at St Michael's Hospital in Bristol was between 1985 and 1995. The clinic is run as a subspecialty service in a university teaching hospital, dealing with infertile couples referred by GPs in the Bristol area and providing tertiary care to couples referred by specialists in Bristol, the South West of England and parts of South Wales. In this cross-sectional study, all women included had a diagnostic laparoscopy for assessment of tubal patency, fibrosis, distortion, or the presence of endometriosis or pelvic adhesions. Women who had a distinct cause of infertility such as ovulatory dysfunction with no index of pelvic disease would not have had a routine laparoscopy and some others

Table I. Characteristics of the infertile women undergoing laparoscopy studied in relation to median CATs

		n (%)	Median CAT[a]	Probability
Female infertility	Secondary	475 (47.5%)	1:128	$P < 0.001$
	Primary	524 (52.4%)	<1:64	
Couple's infertility	Secondary	271 (27.3%)	1:64	$P = 0.7$ (NS)
	Primary	724 (72.7%)	1:64	
Smoker	Yes	361 (35.9%)	1:512	$P < 0.001$
	No	645 (64.1%)	<1:64	
Previous history of PID	Yes	68 (6.8%)	1:1024	$P < 0.001$
	No	938 (93.2%)	1:64	
Tubal damage or pelvic adhesions present	Yes	434 (43.1%)	1:512–1:1024	$P < 0.001$
	No	572 (56.9%)	1:64	

The differences in CAT were compared using the Kruskal–Wallis test.
[a]Range <1:64 to ⩾1:4096 in all subgroups.
PID = pelvic inflammatory disease.

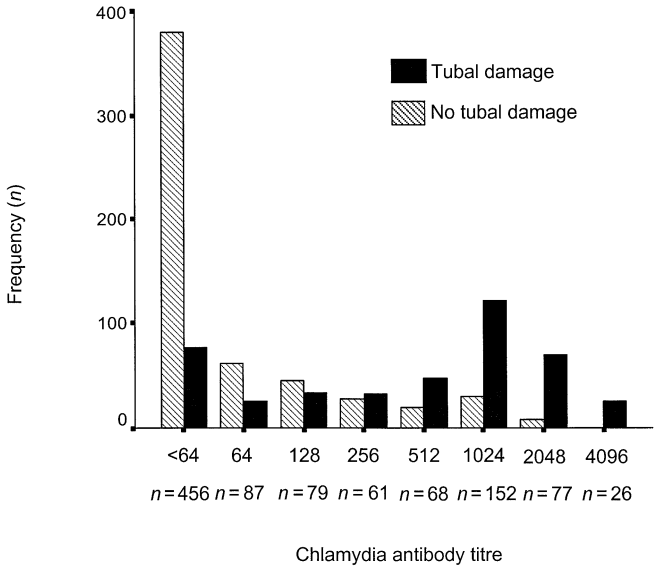

Figure 1. Frequency (*n*) distribution of chlamydia antibody titres in women with and without tubal damage undergoing diagnostic laparoscopy for infertility.

conceived before laparoscopy was necessary or arranged. Although different clinicians carried out the laparoscopies over this period of time, each clinician employed the same technique because they were supervised initially by one of two consultants (Professor M.G.R.Hull or Mr P.G.Wardle) prior to being allowed to assess the pelvis independently. All the clinicians were accredited specialists or senior trainees. Findings were recorded in a standardized way. Once laparoscoped, all women were studied, regardless of infertility diagnosis. A diagnosis of pelvic endometriosis was made if there were any typical superficial lesions or higher grade disease.

Infertility was defined as secondary if a previous conception had occurred, regardless of the outcome. A history of pelvic inflammatory disease (PID) was defined as a previous acute episode of pelvic infection. Smoking implied that the women were smokers at the time of consultation. If there was more than one apparent cause of infertility, priority was given to the condition that was more likely to be the immediate cause. Women with tubal damage (or pelvic adhesions not due to endometriosis) served as the 'cases' to be identified by the test (chlamydia serology) and women without damage served as 'controls' regardless of their other infertility diagnoses. Women were also categorized according to three main findings at laparoscopy: (i) tubal damage; (ii) endometriosis; or (iii) normal pelvis. Tubal damage was diagnosed by the finding of tubal occlusion, and/or distortion of the fimbriae, and/or restrictive tubal–ovarian adhesions, in the absence of visible endometriosis. Severe tubal damage was classified using the 'Hull and Rutherford' classification for tubal disease (Akande, 2002; Rutherford and Jenkins, 2002). This classification referred to women with bilateral tubal damage with extensive tubal fibrosis, and/or tubal distension >1.5 cm, and/or an abnormal tubal mucosal appearance and/or bipolar occlusion, and/or extensive dense pelvic adhesions.

Laboratory procedures

A clotted blood sample was obtained from the patient prior to the laparoscopy and sent to the Public Health Laboratory Service in Bristol for assay. Both clinical and laboratory personnel were blind as to the pelvic status of the woman at that time. Serum samples were assayed for chlamydia IgG antibody employing the single-antigen inclusion test using indirect immunofluorescence, as previously described by Richmond and Caul (1975), otherwise known as the whole-cell inclusion immunofluorescence (WIF) assay. This was applied in practice (Conway *et al.*, 1984) and modified using *C.trachomatis* L2 serotype (Saikku and Paavonen, 1978) as antigen to infect McCoy cell monolayers and anti-human IgM–IgA–IgG–fluorescein conjugate. Dilutions of sera were expressed as antibody titres from 1:64 to ⩾1:4096, or negative (<1:64) (Chernesky *et al.*, 1998).

Statistical analysis

Statistical analysis included non-parametric Spearman's rank correlation coefficients (r_s) and Mann–Whitney *U*-tests (to compare titres between pairs of groups), χ^2-tests of trend and standard measures of diagnostic power.

Results

A total of 1119 infertile women (of all diagnostic groups) who underwent laparoscopic investigation for infertility were identified. Complete data including chlamydia serology were available for 1006, and subsequent analysis is based on these. The women's ages ranged from 18 to 48 years (median 31 years). There was a significant negative correlation [r_s = –0.175, 95% confidence interval (CI) –0.234 to –0.114; $P < 0.0001$] between CAT and age. The duration of infertility at the time of laparoscopy ranged from 1 to 20 years (median

Table II. Number of women in relation to main diagnosis causing infertility and frequency of tubal damage and pelvic adhesions found at laparoscopy

Main cause of infertility	Frequency of diagnosis	Women with tubal damage or pelvic adhesions found at laparoscopy
Tubal damage	390 (38.8%)	390
Sperm dysfunction	181 (18.0%)	29
Unexplained	132 (13.1%)	0
Endometriosis (all grades)	222 (22.2%)	10
Ovulatory dysfunction	29 (2.9%)	2
Cervical mucus dysfunction	11 (1.1%)	2
Tubal sterilization	38 (3.8%)	1
Large fibroids with uterine distortion	3 (0.3%)	0
Total	1006 (100.0%)	434

3.8 years). It was not significantly correlated with CAT and there was no significant difference in the duration of infertility between women with or without tubal damage.

CATs were significantly higher in women who had conceived previously compared with those with primary infertility. However, there was no significant difference between titres according to the couples' past history of pregnancy. Smokers were found to have significantly higher titres than non-smokers (Table I). Although <7% of the women revealed a past history of PID, CATs in these women were significantly higher than in those with no previous history (Table I).

The antibody titres in women with tubal damage were significantly higher than in women without tubal damage ($P < 0.001$; Table I) as also evident in the frequency distribution of women with and without damage shown in Figure 1. Table II shows the diagnostic groups of the women in the study. Three hundred and ninety women had tubal damage as their main cause of infertility, and 44 women had tubal damage as an additional cause (secondary diagnosis), so that a total of 434 of the 1006 women (43%) had tubal damage or pelvic adhesions not related to endometriosis on the basis of laparoscopic findings. Of the 434 women with tubal damage, 417 (96%) had some form of pelvic adhesions and 296 (68%) had at least one tube occluded. Women with tubal damage but no tubal occlusion had significantly lower median antibody levels than those with at least one tube occluded (1:512 versus 1:1024; $P < 0.001$).

The distribution of antibody titres for all women and according to pelvic findings at laparoscopy is shown in Figure 2. The antibody titres for all women with endometriosis ($n = 252$, i.e. $n = 222$ as primary infertility diagnosis and $n = 30$ as secondary diagnosis) were examined separately to determine the association between the adhesions found in women with endometriosis and evidence of previous chlamydial infection. This figure illustrates that titres were significantly higher in women with tubal damage compared with those with a normal pelvis or endometriosis ($P < 0.001$, Kruskal–Wallis test). However, if severe tubal damage was found ($n = 225$) then their titres were highest, and significantly higher than those with less severe damage ($P < 0.001$, data not shown).

A linear relationship between serum CAT and the likelihood of tubal damage was observed (Figure 3). The trends of increasing chlamydia antibody levels in relation to frequency of any tubal damage or severe tubal damage were both highly significant ($P < 0.001$; χ^2 for trend). Figure 3 clearly illustrates that 17% (95% CI 13–20%) and 6.1% (95% CI 4.1–8.7) of women with negative titres (<1:64) had tubal damage or severe tubal damage, respectively. In women with the highest titres of ⩾1:4096, 100% (95% CI 87–100%) had tubal damage while 73.1% (95% CI 52.2–88.4%) had severe tubal damage. This diagram also illustrates that at higher titres, a greater proportion of women are likely to have severe tubal damage than at lower titres. The diagnostic ability of the immunofluorescence test used in detecting women with any degree of tubal damage and severe tubal damage is shown in Table III. Increasing the cut-off level improved specificity and the positive likelihood ratio (LR+) at the expense of sensitivity and the negative likelihood ratio (LR–).

Discussion

This is the first report of its kind employing a large population of infertile women for comparative analysis. Our study using laparoscopy on all patients confirms that past infection with *C.trachomatis* is associated with a significantly increased risk of women suffering tubal infertility, as shown by others (Conway *et al*., 1984; Westrom, 1987; Forsey *et al*., 1990; Mol *et al*., 1997; Thomas *et al*., 2000; Veenemans and van der Linden, 2002). Negative chlamydia serology (<1:64) does not, however, preclude the diagnosis of tubal damage. Conversely, high titres do not necessarily indicate the presence of tubal damage, as shown by the high titres observed in some women with a normal pelvis in the present study.

Laparoscopy is the accepted gold standard for the diagnosis of tubal damage (RCOG, 1998). The high prevalence of tubal damage observed may reflect the prolonged duration of infertility (3.8 years) of the women studied. The present study, however, was on a select group of women undergoing detailed infertility investigations in a tertiary setting. The studies' use of controls from non-tubal cases theoretically meets the requirement for controls. Even so, there is a possibility that excluded cases who were not laparoscoped had conditions which precluded the use of laparoscopy or indeed that many minor cases of tubal damage may not have been picked, as they became pregnant spontaneously prior to laparoscopy. Nonetheless, a significant proportion of patients who underwent laparoscopy had no pelvic damage and were infertile due to other causes such as sperm or ovulatory dysfunction and unexplained infertility (Figure 2 and Table II).

The relatively high sero-prevalence of positive CAT and the relatively low proportion of women who give a history of previous PID attest to chlamydial infection being mainly asymptomatic (Bjercke and Purvis, 1993; Stamm, 1999). Smokers were also more likely to have a higher CAT, which may be due to socio-economic and other risk factor associations (Shafer *et al*., 1993). Ectopic pregnancies are a reflection of tubal damage, and smoking is recognized to be an independent risk factor (Saraiya *et al*., 1998; Bouyer *et al*.,

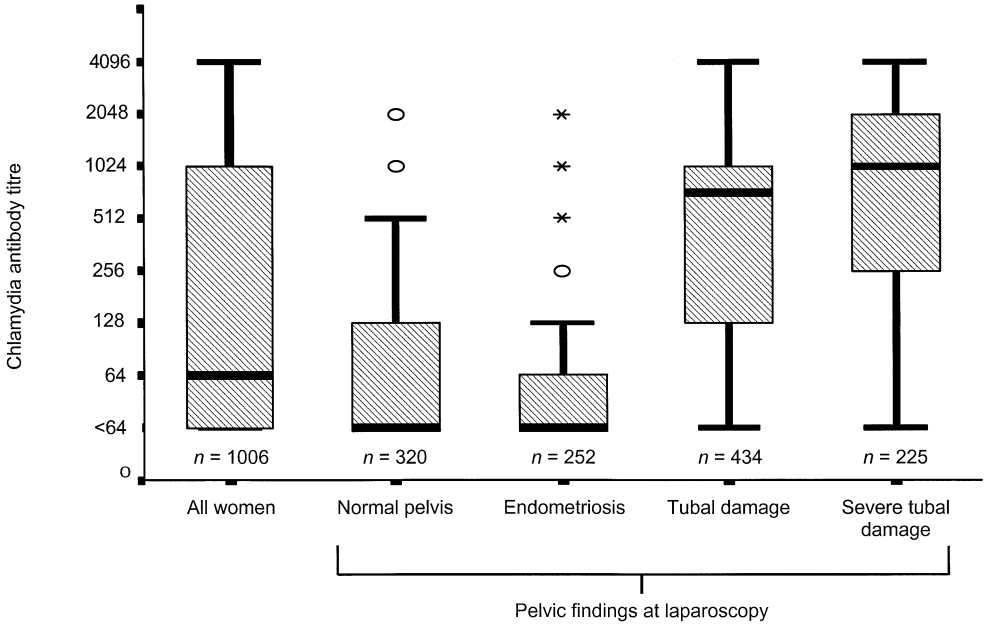

Figure 2. Chlamydia antibody titres for all infertile women studied and according to pelvic findings at laparoscopy (horizontal lines = medians; shaded boxes = quartiles; whiskers = ranges; ovals = outliers; * = extremes).

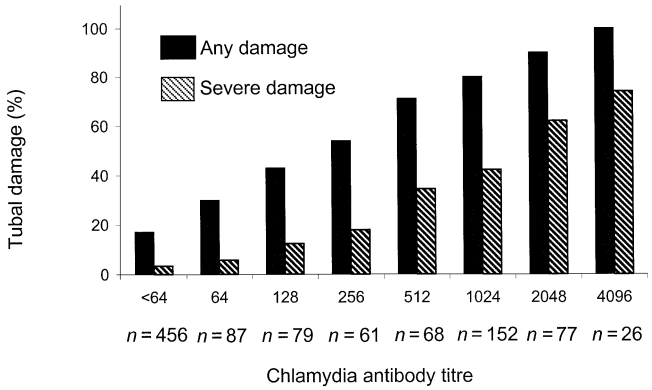

Figure 3. Frequency (%) of any tubal damage or severe tubal damage according to chlamydia antibody titres.

2003), as is previous chlamydial infection (Brunham *et al*., 1986; Sheffield *et al*., 1993). It is therefore plausible that smoking predisposes women to tubal damage and thus ectopic pregnancies via increased susceptibility to chlamydia infection; this however remains to be tested.

The sensitivity of chlamydia serology in detecting tubo-peritoneal damage has been demonstrated by others (Dabekausen *et al*., 1994; Meikle *et al*., 1994; Land *et al*., 1998), including a meta-analysis (Mol *et al*., 1997). However, these studies included women with endometriosis considered as positive cases. The present study found that the majority of women with endometriosis had negative titres (median CAT <1:64), implying that adhesions found at laparoscopy in these women are unlikely to be of infective origin and therefore are unlikely to be detected using chlamydia serology. Like Anestad *et al*. (1987), we found that pelvic adhesions (not due to endometriosis) were the most frequent sequelae associated with a high CAT. The findings of their study (Anestad *et al*., 1987) suggested that adhesions were the most likely consequence of chlamydial infection, with occlusion being a manifestation of more severe infection associated with higher titres, consistent with our findings. chlamydia and gonorrhoea are both common causes of PID and often co-exist (Rice and Schachter, 1991), It is therefore plausible that in the women who had a history of acute PID and had negative chlamydia serology, this was caused by gonorrhoea or other organisms (Clausen *et al*., 2001).

A meta-analysis (Mol *et al*., 1997) showed that the performance of chlamydia antibody testing depended on the assay used, and found the WIF test with the enzyme-linked immunosorbent assay (ELISA) and micro-immunofluorescence (MIF) test to be superior to the immunoperoxidase assay. However, the studies examined were not strictly comparable because some were based on tubal damage diagnosed by HSG alone, and non-uniform cut-off levels were used. The immunofluorescence test employed in the present study is highly sensitive, as shown by a blinded comparative study of other serological tests for *C.trachomatis* antibody carried out in three international centres including one of ours (Chernesky *et al*., 1998; Jones *et al*., 2003). The test has the ability to detect both group lipopolysaccharide and species-specific antibodies, therefore high titres are indicative of highly specific *C.trachomatis* species antibody (Black, 1997). Consequently, women with positive serology but with a normal pelvis may have had non-genital chlamydia infection. In these cases, cross-reactive responses to past infection with other species of chlamydia such as *Chlamydia pneumoniae* (Gijsen *et al*., 2001) or *Chlamydia psittaci* (Bergstrom *et al*., 1996; Jones *et al*., 2003) is a possibility, but difficult to account for. Time-related antibody titre decline is a possible reason for false

Table III. Diagnostic test analysis for all women with any tubal damage and pelvic adhesions and for women with severe tubal damage and pelvic adhesions in relation to serum CAT (immunoflouresence test) amongst infertile women undergoing laparoscopy for infertility (n = 1006)

CAT	True pos	True neg	False pos	False neg	Sensitivity (%)	Specificity (%)	Pos PV(%)	Neg PV (%)	Pos LR	Neg LR
Women with any tubal damage (n = 434)										
⩾1:64	358	380	192	76	82	66	65	83	2.5	0.26
⩾1:128	332	441	131	102	76	77	72	81	3.3	0.30
⩾1:256	298	486	86	136	69	85	78	78	4.6	0.37
⩾1:512	265	514	58	169	61	90	82	75	6.0	0.43
⩾1:1024	217	534	38	217	50	93	85	71	7.5	0.54
⩾1:2048	95	564	8	339	22	99	92	62	15.6	0.79
⩾1:4096	26	572	0	408	6	100	100	58	–	0.94
Women with severetubal damage[a] (n = 225)										
⩾1:64	197	428	353	28	88	55	36	94	1.9	0.23
⩾1:128	188	506	275	37	84	65	41	93	2.4	0.25
⩾1:256	174	571	210	51	77	73	45	92	2.9	0.31
⩾1:512	159	617	164	66	71	79	49	90	3.4	0.37
⩾1:1024	137	663	118	88	61	85	54	88	4.0	0.46
⩾1:2048	67	745	36	158	30	95	65	83	6.5	0.74
⩾1:4096	19	774	7	206	8	99	73	79	9.4	0.92

[a]Severe tubal damage: bilateral tubal damage with extensive tubal fibrosis and/or tubal distension and/or abnormal tubal endosalpinx and/or bipolar tubal occlusion and/or extensive dense adhesions.
Pos = positive; neg = negative; PV = predictive value; LR = likelihood ratio.

negatives (i.e. negative serology but positive laparoscopy), but this issue may be controversial. Previous studies have suggested a chronological decline in titres (Puolakkainen *et al*., 1986; Henry-Suchet *et al*., 1994); however, a more recent study revealed no significant decline (Gijsen *et al*., 2002). Another explanation for false negatives may be that the immune-mediated reaction responsible for adhesions or tubal occlusion may not have occurred in these women for unknown reasons (Witkin *et al*., 2000). Nonetheless, it is recognized that the *C.trachomatis* organism can be found in apparently healthy normal-looking Fallopian tubes (Menchaca *et al*., 1988; Shepard and Jones, 1989; Marana *et al*., 1990; Stacey *et al*., 1990). This suggests that its presence in the genital tract does not always result in damage and is consistent with the findings of the present study.

Because there are justified constraints to the indiscriminate use of laparoscopy and HSG, there is a need to minimize the number of patients subjected to these diagnostic investigations who do not have disease (false positives). Diagnostic tests have three main uses: diagnosis of disease; screening; and patient management (Campbell and Machin, 1993). Screening is defined as a procedure that helps identify a specified disease or condition (in this context, tubal damage). As most screened individuals will be unaffected, the test must be safe and acceptable. As a screening test, chlamydia serology would also be allowed to possess higher margins of error and may be less accurate than diagnostic tests (Peters *et al*., 1996). If laparoscopy is readily available and the primary aim of screening is to avoid delay in referral for IVF or tubal surgery in those with significant tubal damage, false negatives have to be minimized. As such, a low cut-off may be the preferred option in view of its higher sensitivity. To achieve the objective of identifying a subgroup of infertile women for further investigation, a cut-off level is required. However, a universal single cut-off which splits women into two groups is likely to be controversial.

Approaches to dealing with the problem of a cut-off titres have to take into account the quantitative nature of serology in relation to frequency of disease. For each antibody titre, the sensitivity, specificity, and positive and negative likelihood ratios should be calculated (Land *et al*., 1998; Akande *et al*., 2002). These parameters can be used to dichotomize patients into those that are high risk and low risk of being found with tubal damage. However, in defining a cut-off titre, three main issues need to be considered (Coggon *et al*., 1993). First, a cut-off titre based on statistical analysis which is acceptable as a simple guide to the limits of what is common. Secondly, a cut-off titre based on the perceived or actual clinical importance may define the level of the antibody titre above which the finding of tubal damage becomes more frequent. Finally, a cut-off based on prognostic factors where high chlamydia antibody levels may be associated with a greater severity of disease and a lower chance of conception. Each of these approaches is suitable for different purposes and needs to be defined when being used (Coggon *et al*., 1993). However, it is not always necessary to state a value as the need to do so would depend on whether the aim is to make a diagnosis or a prognosis (Altman, 1991). Again, this is a clinical judgement and not a statistical issue. For example, in screening for lethal disease, high sensitivity is desirable though the trade off is usually lower specificity. However, when dealing with a non-life-threatening condition such as tubal damage, a high cut-off (lower sensitivity) may be chosen, but this may miss some cases but lead to fewer women who do not have the disease being subjected to invasive and costly laparoscopy (high specificity). The use of prevalence-independent likelihood ratios can also help overcome this problem; a suitable cut-off titre would have a positive likelihood ratio >5 and a negative likelihood ratio <0.2 (Land *et al*., 1998). Although the choice of cut-off level is discretionary, our findings (based on the likelihood ratios) suggest that with a titre of ⩾1:256 using the WIF test, the risk of a woman having tubal damage is increased sufficiently to

consider early referral for further investigation. Titres <1:256 may justify early referral only if there were other risk factors such as previous abdominal surgery or history of pelvic infection and the couples had not conceived within a reasonable amount of time.

It is tempting to suggest that early detection of a disease is an end in itself. However, the spectrum of disease varies according to the severity and extent of lesions (Akande, 2002). The present study is clear in demonstrating that severe damage is more likely in women with higher titres. This implies that increasing antibody titres are quantitatively related to both the presence of tubal damage and the severity of tubal damage. Thus identification of trivial disease such as minor filmy adhesions or indeed untreatable conditions such as bilateral distended hydrosalpinges are important in terms of prognosis for fertility for different reasons (Akande, 2002). Consequently, identifying women who are at sufficiently high risk of having severe tubal damage impairing fertility may be more important than identifying women with minimal tubal damage. In these circumstances, a higher antibody titre cut-off would be required (Table III).

Striking a balance, in a target population between, on the one hand, the severity of the disorder affecting fertility and the prevalence of disease, and, on the other, the availability, costs, hazards and acceptability of invasive diagnosis is a practical necessity. This study shows that using chlamydia serology for screening provides a useful guide to the risk of tubal damage causing infertility but also exposes certain limitations of this method of screening. However, the choice of cut-off level used for screening would depend on the prevalence of the disease in the target population to which it is applied and whether one wants to identify most cases of women with tubal damage or mainly those with severe damage.

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