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Quantitative study of Chlamydia trachomatis in genital infection

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SUMMARY *Chlamydia trachomatis* inclusion counts on inoculated McCoy cell coverslips were used as an index of the degree of infection of the cervix in women and of the urethra in men with urethritis. High inclusion counts were obtained significantly more often from men than from women, from women with cervical ectopy, and from women who had had recent sexual intercourse. Low inclusion counts were significantly more common in men with a past history of gonococcal urethritis.

Higher chlamydial isolation rates in women with gonorrhoea and in women taking the contraceptive pill could not be attributed to a greater degree of infection, since inclusion counts were not raised in these patients. There was evidence that strains of *C trachomatis* might vary in their ability to establish themselves in the genital tract because high counts in men with NGU were associated with high counts in their female consorts and the levels of counts in men were associated with the frequency of chlamydial isolation from their female consorts.

The relatively simple technique of inclusion counts in cultures for chlamydia from the genital tract may yield valuable information about the behaviour of different strains of *C trachomatis* in causing pathological changes, in the transmission of infection between individuals, and in the response to specific chemotherapy.

Introduction

When *Chlamydia trachomatis* is grown in McCoy cell tissue culture the number of intracellular inclusion bodies that develop after 2-3 days directly reflects the number of viable particles in the original inoculum. Inclusion counts in cultures from the genital tract may thus be used to assess the degree of infection.[1] In a survey of patients attending a sexually transmitted diseases (STD) clinic we have graded chlamydia-positive cultures into those with low, high, or very high inclusion counts and related these to epidemiological and clinical aspects of the infections.

Patients and methods

STUDY POPULATION

This comprised all men with urethritis and all women who attended the STD clinic at St James's Hospital, Birkenhead, on Monday afternoons or Thursday mornings from January 1976 to September 1978. Patients who had received an antibiotic or sulphonamide in the past month and men who had reported urethritis within the past three months were excluded.

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CLINICAL AND CULTURAL TECHNIQUES

Demographic data, past history, history of recent exposure, symptoms, and signs were recorded on precoded proformas. Details of the examination of female patients and cultural methods, including the isolation of *C trachomatis* on idoxuridine-treated McCoy cell coverslips, have been described.[2,3] Urethral swabs from men were cultured for *Neisseria gonorrhoeae* and *C trachomatis* and used to prepare smears for identifying pus cells and Gram-negative intracellular diplococci. Urethritis was diagnosed when smears showed $\geqslant$15 polymorphonuclear leucocytes (PMNL) in each of five microscope fields ($\times$ 100 objective, $\times$ 6·3 eye-pieces).

CHLAMYDIAL INCLUSION COUNTS

Chlamydia-positive coverslips (12mm in diameter) were graded as showing low or high inclusion counts

by scanning across several diameters with the × 20 objective and × 10 eye-pieces of the dark-ground microscope. Under these conditions each traverse of a coverslip diameter covered one twenty-fourth part of the whole coverslip area.

Low counts: Less than 5 inclusions per diameter was equivalent to <120 inclusions per whole coverslip; such coverslips were graded as giving low inclusion counts.

High counts: Coverslips with ⩾5 inclusions per diameter (⩾120 inclusions per coverslip) were graded as giving high inclusion counts.

Very high counts: Some coverslips had many inclusions in every microscope field (one nine-hundredth of the coverslip area); those with ⩾5 inclusions per field were graded as giving very high (⩾4500) inclusion counts.

STATISTICAL ANALYSIS

The data were analysed with a computer using the 'statistical package for social sciences'. Tests of significance were performed by the χ^2 method applying Yates's correction where appropriate.[4]

Results

C trachomatis was isolated from 147 of 474 (31%) women and from 191 of 413 (46%) men with urethritis. Inclusion counts were performed on a random selection of 115 women and 181 men (tables I and II). Overall, high counts (⩾120) were found in about half (156 or 53%) and very high counts (⩾4500) in almost one-fifth (52 or 18%) of these 296 positive coverslips. Swabs with high counts were obtained significantly more often from the chlamydia-positive men with urethritis (57%) than from the chlamydia-positive women (45%) ($\chi^2_1 = 4 \cdot 23$, $P < 0 \cdot 05$).

WOMEN

The distribution of inclusion counts was not affected by age, marital state, or stage of menstrual cycle.

Although isolation rates for chlamydia were significantly higher ($\chi^2_1 = 13 \cdot 3$, $P < 0 \cdot 001$) in those using oral contraceptives (40% chlamydia-positive) than in those not practising contraception (21% chlamydia-positive) the use of oral contraceptives did not influence the incidence of high inclusion counts. An

TABLE I *Factors studied in relation to C trachomatis inclusion counts in women*

Factor examined	*Total No of patients*	*No (%) of patients with following inclusion counts:*					
		Low (<120)		*High (⩾120)*		*Very high (⩾4500)*	
All women	115	63	(55)	52	(45)	17	(15)
Age (years)							
15-19	33	19	(58)	14	(42)	6	(18)
20-29	68	36	(53)	32	(47)	9	(13)
⩾30	14	8	(57)	6	(43)	2	(14)
Contraceptive method							
Pill	82	44	(54)	38	(46)	11	(13)
None	21	13	(62)	8	(38)	3	(14)
Last intercourse							
<1 week	37	12	(32)	25	(68)	8	(22)
1-2 weeks	53	29	(55)	24	(45)	8	(15)
⩾3 weeks	25	22	(88)	3	(12)	1	(4)
Cervical ectopy							
Absent	29	21	(72)	8	(28)	2	(7)
Simple	43	24	(56)	19	(44)	5	(12)
Hypertrophic	43	18	(42)	25	(58)	9	(21)
T vaginalis							
Present	18	14	(78)	4	(22)	2	(11)
Absent	97	49	(51)	48	(49)	15	(16)
Candidosis							
Present	19	11	(58)	8	(42)	3	(16)
Absent	96	52	(54)	44	(46)	14	(15)
Gonorrhoea							
Present	47	25	(53)	22	(47)	6	(13)
Absent	68	38	(56)	30	(44)	11	(16)
Cervical mucopus:							
With gonorrhoea							
Present	13	4	(31)	9	(69)	2	(15)
Absent	34	21	(62)	13	(38)	4	(12)
Without gonorrhoea							
Present	17	9	(53)	8	(47)	3	(18)
Absent	51	29	(57)	22	(43)	8	(16)
Residual symptoms after treatment for gonorrhoea							
Present	12	5	(42)	7	(58)	3	(25)
Absent	32	18	(56)	14	(44)	4	(13)

TABLE II *Factors studied in relation to C trachomatis inclusion counts in men with urethritis*

Factor examined	Total No of patients	No (%) of patients with following inclusion counts: Low (<120)		High (≥120)		Very high (≥4500)	
All men with urethritis	181	77	(43)	104	(57)	35	(19)
Age (years)							
15-19	22	5	(23)	17	(77)	7	(32)
20-29	108	51	(47)	57	(53)	19	(18)
≥30	51	21	(41)	30	(59)	9	(18)
Men with							
GU	45	22	(49)	23	(51)	6	(13)
NGU	136	55	(40)	81	(60)	29	(21)
Past history of NGU*							
Present	53	24	(45)	29	(55)	10	(19)
Absent	127	53	(42)	74	(58)	25	(20)
Past history of GU*							
Present	43	24	(56)	19	(44)	3	(7)
Absent	137	53	(39)	84	(61)	32	(23)
Men with NGU whose consorts were:							
Chlamydia −	14	9	(64)	5	(36)	1	(7)
Chlamydia + (<120)	9	4	(44)	5	(56)	2	(22)
Chlamydia + (120-4500)	8	3	(38)	5	(62)	3	(38)
Chlamydia + (>4500)	5	1	(20)	4	(80)	2	(40)

+ Positive; − negative; NGU = non-gonococcal urethritis; GU = gonococcal urethritis
* Information not recorded for one patient

effect of recent intercourse was seen in that high counts ($\chi^2_1 = 11 \cdot 0$, $P < 0 \cdot 001$) or very high counts ($\chi^2_1 = 4 \cdot 21$, $P < 0 \cdot 05$) were obtained significantly more often compared with low counts in those who reported intercourse within the previous week.

High counts were significantly associated with the presence of ectopy ('erosion') ($\chi^2_1 = 3 \cdot 96$, $P < 0 \cdot 05$), with very high counts being found more frequently in patients whose ectopy was hypertrophic.

Low counts were more common in those with concurrent *Trichomonas vaginalis* infection than in those without ($\chi^2_1 = 3 \cdot 51$, $P > 0 \cdot 05$) but counts were not affected by concurrent *Candida* infection. Although isolation rates for chlamydia were significantly higher ($\chi^2_1 = 16 \cdot 2$, $P < 0 \cdot 001$) in those with gonorrhoea (44% chlamydia-positive) than in those without (25% chlamydia-positive), the distribution of inclusion counts was unaffected by concurrent gonococcal infection. In women with gonorrhoea, however, cervical mucopus was associated with high counts, but these were not found in the presence of mucopus without gonococcal infection. Women who complained of persistent residual symptoms after treatment for gonorrhoea included more with high counts initially than those in whom symptoms resolved quickly.

MEN

High counts were more common in men under 20 years than in those over, but the difference was not significant ($\chi^2_1 = 3 \cdot 16$, $P > 0 \cdot 05$). In men with non-gonococcal urethritis (NGU) high and very high counts were more frequent than in those with gonococcal urethritis (GU) but the difference was not significant. In both groups a past history of NGU did not affect the distribution of counts but a history of GU was associated with a reduction in the frequency of high counts ($\chi^2_1 = 3 \cdot 91$, $P < 0 \cdot 05$) or very high counts ($\chi^2_1 = 4 \cdot 61$, $P < 0 \cdot 05$).

In men with NGU, high counts were associated with high counts in their female consorts. There was also a link between counts in men and the frequency of chlamydial isolation from their female consorts. Of eight men with very high counts, seven (88%) had consorts infected with *C trachomatis;* of 11 men with high but not very high counts, seven (64%) had infected consorts; of 17 men with low counts, eight (47%) had infected consorts.

Discussion

Chlamydia trachomatis is an organism that is commonly transmitted by sexual intercourse and from mothers to new-born infants. It causes urethritis in men, conjunctivitis and pneumonitis in babies, and infection of the genital tract in women which is often symptomless and not associated with any detectable pathological lesion. It is clearly important to establish whether the numbers of chlamydia present in various infected or carrier sites have a bearing on the pathology and epidemiology of *C trachomatis* infection.

Hilton *et al*[5] found that women taking oral contraceptives had a significantly higher proportion of specimens with more than 100 inclusions per coverslip than did women not taking the contraceptive pill. In a study of women consorts of men

with NGU, Tait *et al*[6] found higher mean inclusion counts to be associated with the use of oral contraceptives or the presence of endocervical mucopus but not with hypertrophic ectopy. In a much larger study, however, Hobson *et al*[1] confirmed the association of high counts (>1000) with cervical mucopus but found in contrast that high counts were associated with the presence of cervical ectopy but not directly with the use of the contraceptive pill. Our present results support this latter finding of high counts in those with cervical ectopy and a lack of effect on the count of the contraceptive pill. The tendency to find high counts in those with cervical mucopus was apparent only in women with gonorrhoea. A reported association of high counts with younger age in women[1] was not confirmed in this study, although a similar trend was suggested in men with urethritis. The significantly higher chlamydial isolation rates in women with gonorrhoea and those taking the contraceptive pill could not be attributed to a greater degree of infection since inclusion counts were not found to be raised in these patients.

In men it is not clear why fewer high counts were obtained from patients with a history of gonococcal but not non-gonococcal urethritis. There was an association between high counts in men and in their female consorts and between inclusion counts in men and the frequency with which chlamydia were isolated from their female consorts. This suggests that some strains of *C trachomatis* may establish themselves in the genital tract more readily than others and this may be a factor determining the numbers of organisms present.

The estimation of inclusion counts would be unlikely to improve the routine management of individual patients with STD. A knowledge of both isolation rates and the distribution of inclusion counts in a patient population should, however, allow a clearer understanding of *C trachomatis* infections. It may yield valuable information on the effect of strain variation in causing pathological changes in the genital or respiratory tracts, on the transmission of infection from one individual to another, on the response to specific chemotherapy, and on the relation to other features in the genital tract, such as associated infections including gonorrhoea, trichomoniasis, and candidosis.

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