

CERVICAL CHLAMYDIA TRACHOMATIS AND MYCOPLASMAL INFECTIONS IN WOMEN WITH ABNORMAL PAPANICOLAOU SMEARS

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Summary: In a series of 2,346 Papanicolaou-stained smears from women undergoing routine gynaecological examination, 39 showed cytomorphological signs of inflammation suggesting Chlamydia trachomatis infection (Papanicolaou class II or III). The 39 smears were studied microbiologically by the direct-immunofluorescence test and cell culture to see whether chlamydial infection correlated with the presence of Mycoplasma hominis and Ureaplasma urealyticum. The results were compared with the cytological and colposcopic findings. C. trachomatis was cultured in 56.41% of the 39 smears, and isolated by the direct-immunofluorescence test in 51.28%. M. hominis was detected in 35.89% and U. urealyticum in 25.54%. Though all three organisms coexisted in 10.25% of the smears, C. trachomatis and M. hominis in 15.38%, C. trachomatis and U. urealyticum in 2.56%, no valid conclusions could be drawn from their association. The study did, however, indicate that vacuolated cells and cells with "bubbly" cytoplasm are common also to other infections seen in PAP-test smears and do not necessarily warrant a diagnosis of C. trachomatis, but that Gupta-type intracellular inclusion bodies do.

INTRODUCTION

In the identification of patients whose cervical smears warrant further microbiological studies, Papanicolaou staining is still valid⁽⁷⁾. These smears often display cell abnormalities that are etiologically hard to explain: vacuolated cytoplasm, typical chlamydial intracytoplasmic inclusions, koilocytes and the keratocytes seen in Papilloma virus infection (HPV), all accompanied or not by varying degrees of dysplasia. Recent studies suggest that Chlamydia trachomatis (Ct), a sexually transmitted agent, can be associated with disease of the uterine cervix, such as cervicitis⁽³⁾ or dysplasia^(6, 16, 18). The infection may migrate from the lower genital tract back to the endometrium causing endometritis⁽²⁵⁾, salpingitis, oophoritis or pelvic inflammatory disease (PID), and tubal damage or ectopic pregnancy, possibly with resultant infertility^(13, 14, 22). Ct infection is also exacerbated by obstetric trauma, insertion of intrauterine devices

and salpingography⁽²⁴⁾. Most cases of nongonococcal urethritis and acute epididymitis in men are caused by Ct⁽²¹⁾. Infants, who acquire the infection from the mother through the birth canal, frequently develop Ct conjunctivitis or interstitial pneumonia in the early months of life⁽¹⁹⁾.

Mycoplasma hominis, another organism often isolated from the cervical canal, provokes retrograde genital infections including acute salpingitis, endometritis and PID⁽¹⁵⁾. The infection spreads by the same route as Ct, to the upper reproductive tract, via the blood or lymph vessels and is facilitated by lesions of the uterine cervix or endometrium from interruptions of pregnancy or other gynaecological procedures⁽¹⁴⁾. As a result of infection, the newborn may develop conjunctivitis, cerebral abscess, or meningitis⁽¹¹⁾.

The microorganism Ureaplasma urealyticum is also frequently isolated from the uterine cervix. It may be associated with recurrent spontaneous abortion^(2, 23) and infertility of unknown origin⁽¹⁴⁾; as the

cause of chorio-amnionitis and pneumonia it markedly affects perinatal morbidity and morbidity⁽¹⁷⁾.

Harrison considers that a finding of one of these affections may suggest the presence of the other two. Their coexistence has been noted mainly in pregnant women⁽¹¹⁾ but no published studies have investigated the meaning of this phenomenon in other genital tract diseases. In this study we isolated Ct, *M. hominis* and *U. urealyticum* in Papanicolaou-stained smears selected for further study because of epithelial cell changes indicative of Ct or HPV infection, sometimes associated with dysplasia. The aim was to confirm the presence of Ct by cultures and direct-immunofluorescence tests, evaluate the utility of cytology in detecting chlamydial infections, study the possible relationship between Ct, *M. hominis*, *U. urealyticum* and uterine cervix disease, and lastly to compare the microbiological findings with the colposcopic and cytomorphologic appearances.

MATERIAL AND METHODS

During routine gynaecological examinations 2,349 cervical smears taken from women between the ages of 17 and 63 for staining by the Papanicolaou method. Thirty-nine smears were selected for further study because of cell abnormalities: metaplastic and squamous cells with acidophilic granular or uniform intracellular inclusions as described by Gupta⁽⁹⁾ and considered diagnostic of Ct infection; metaplastic cells with intracytoplasmic vacuoles or "bubbly" cytoplasm (with or without inclusions); keratocytes, koilocytes and the parabasal cells found in HPV infection, associated or not with varying degrees of dysplasia (CIN I, CIN II, CIN III).

These features appeared in isolation or association. All the patients had clinical signs of drug-resistant cervical inflammation and erosion of the portio. Three had a history of recurrent spontaneous abortion, 1 of primary sterility, 1 of post-abortion endometritis, 2 of cystitis and recurrent urethritis and 8 women had undergone conization for carcinoma of the cervix. The 39 women were recalled for repeat colposcopic examination and endocervical "cytobrush" cytology.

Duplicate specimens were taken for Ct, *M. hominis* and *U. urealyticum*. The specimens were fixed in acetone and frozen at -70°C until required. Ct isolation was attempted both in cultures and by the direct-immunofluorescence test.

Cultures. Cervical swabs for culture were placed in sucrose-phosphate transport medium and inoculated onto cycloheximide-treated McCoy cell monolayers according to the method of Mårdh and Ripa⁽¹³⁾. After incubation at 37°C for 65-72 hours, the monolayers were stained with Giemsa stain.

Direct-immunofluorescence staining. The cervical smears were incubated with 30 μl of a fluorescence-conjugated monoclonal antibody solution that recognizes the 15 serovariants (immunotypes) of Ct. When antibody-stained specimens are examined under the fluorescence microscope at a magnification of 400-600, Chlamydial particles are seen as small apple-green dots on a background of reddish-brown epithelial cells.

Mycoplasma cultures. *M. hominis* and *U. urealyticum* were isolated using standard techniques; the culture mediums contained respectively arginine or urea⁽¹²⁾. The swabs were placed in 14 \times 100 mm vials with rubber stoppers and incubated at a constant temperature of 36.5°C , then passed in Agar. After a mean of 72 hours the mycoplasma were identified by typical colony morphology, *M. hominis* with Diene's staining and *U. urealyticum* with urea reactivity (manganese sulphate, CaCl_2)⁽¹²⁾.

RESULTS

Table 1 compares the cytomorphological, colposcopic and microbiological findings in the 39 cases cytologically selected from the 2,346 PAP-test smears, for further study. Ct infection was identified by culture in 22 (56.41%) of the cases and by the direct-immunofluorescence test using fluoresceine-conjugated monoclonal antibodies, in 20 (51.28%). *M. hominis* was isolated in 14 (35.98%) and *U. urealyticum* in 10 (25.64%). In 4 cases (10.25%), all three microorganisms were present, 6 (15.38%) were positive for Ct and *M. hominis* and 1 (2.56%) for Ct and *U. urealyticum*. Four cultures for *U. urealyticum* were contaminated by *Candida*.

Table 1. – Correlation between cytologic and colposcopic findings and *C. trachomatis*, *M. hominis*, *U. urealyticum* investigation.

Cytologic finding	Colposcopic finding	<i>C. trachomatis</i>		<i>M. hominis</i>	<i>U. urealyticum</i>
		Culture	IF	Culture	Culture
Classical chlamydial inclusions	cervicitis	+	+	–	–
Classical chlamydial inclusions	cervicitis	+	+	–	–
Vacuolated metaplastics	erosive cervicitis	–	–	–	+
Bubbly cytoplasm of metaplastics	erosive cervicitis	+	–	+	contaminated
Classical chlamydial inclusions	erosive cervicitis	+	+	–	–
Vacuolated metaplastics	erosive cervicitis	+	–	+	–
Bubbly cytoplasm of metaplastics	cervicitis	–	+	+	+
Classical chlamydial inclusions	erosive cervicitis	+	–	–	–
Vacuolated metaplastics	cervicitis	+	+	–	–
Intravacuolar bodies	cervicitis	–	–	+	contaminated
Classical chlamydial inclusions	cervicitis	+	+	–	–
Classical chlamydial inclusions	erosive cervicitis	+	+	–	–
Vacuolated metaplastics	erosive cervicitis	–	–	+	contaminated
Vacuolated metaplastics	cervicitis	–	–	+	contaminated
Classical chlamydial inclusions	erosive cervicitis	+	+	+	+
Bubbly cytoplasm of metaplastics	cervicitis	–	–	+	–
Bubbly cytoplasm of metaplastics	erosive cervicitis	–	–	+	–
Classical chlamydial inclusions	cervicitis	+	+	–	–
Koilocytes and CIN I	condyloma	–	+	–	–
Intravacuolar bodies	cervicitis	–	–	+	+
Bubbly cytoplasm of metaplastics	erosive cervicitis	+	+	–	–
CIN III	mosaic	–	+	+	+
Bubbly cytoplasm of metaplastics	erosive cervicitis	+	–	+	–
CIN III	mosaic	+	+	–	+
Koilocytes and CIN I	mosaic	+	–	–	–
CIS	condyloma	+	+	–	–
Koilocytic squamous cells	cervicitis	+	+	–	–
Koilocytic squamous cells	cervicitis	+	+	–	–
Vacuolated metaplastics	cervicitis	+	–	–	–
Koilocytes and CIN I	condyloma	+	+	–	–
Intravacuolar bodies	cervicitis	–	+	–	–
Classical chlamydial inclusions	erosive cervicitis	+	+	–	–
Conization for CIS	–	–	–	–	–
Conization for CIS	–	–	–	–	–
Intravacuolar bodies	cervicitis	–	–	–	–
Vacuolated metaplastics	cervicitis-trichomonas	–	–	+	+
Vacuolated metaplastics	cervicitis	–	–	–	+
Koilocytes and CIN I	condyloma	–	–	–	+
CIN III	mosaicum	+	+	+	+

mild dysplasia = CIN I (cervical intraepithelial neoplasia)

severe dysplasia = CIN III (cervical intraepithelial neoplasia)

carcinoma in situ = CIS

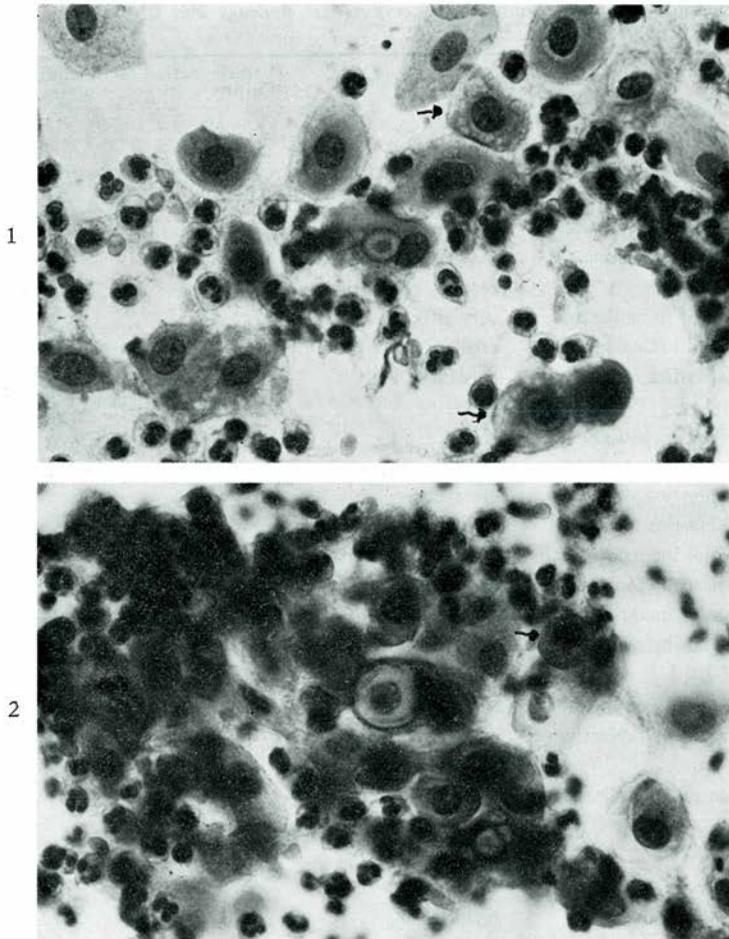


Fig. 1-2. — Classical clamydial inclusions in a patient chlamydia culture positive (Papanicolaou $\times 400$).

DISCUSSION

Controversy surrounds the values of the PAP-test in the diagnosis of Ct infections (4, 6, 7, 8). Gupta (9) describes three recognizable stages of Ct infection in smears: in stage I the cyanophilic cytoplasm contains finely granular coccoid bodies (elementary bodies); in stage II the cytoplasm is finely granular and “bubbly” with intracytoplasmic perinuclear coccoid inclusions (elementary or reticulate bodies); at stage III there are multiple intracyto-

plasmic inclusions with well-defined edges and homogeneous condensation (intermediate bodies, denoting the final stage of the Ct cycle). When inflammation is present, however, other factors may produce various cell changes: vacuolated metaplastic cells, metaplastic cells with “bubbly” cytoplasm, anphophilic intravacuolar inclusion bodies or koilocytic squamous cells. Vacuolated metaplastic cells may mistakenly suggest stage I or II Ct infection, but are also a feature of infection



Fig. 3. — Intravacuolar body in a patient *M. hominis* and *U. Urealyticum* culture positive and chlamydia culture negative (Papanicolaou $\times 400$).

from bacteria such as *M. hominis* and *U. urealyticum*. Especially when associated with parabasal cells with a perinuclear halo, koilocytic squamous and multinucleated cells suggest HPV infection⁽⁵⁾.

We found Gupta-type chlamydial inclusions⁽⁹⁾ in only 9 cases, all of which had positive *Ct* cultures, 1 of these was also positive for both *M. hominis* and *U.*

urealyticum (fig. 1, 2). Smears displaying well-defined intracytoplasmic vacuoles, either empty or filled with dense anphophilic material, were more often positive for *M. hominis* or *U. urealyticum* than for *Ct* (fig. 3). *Ct* was also insolated in 3 patients whose smears contained vacuolated metaplastic cells (fig. 4), in 3 with cytoplasmic “bubbling” and in 2 cases with

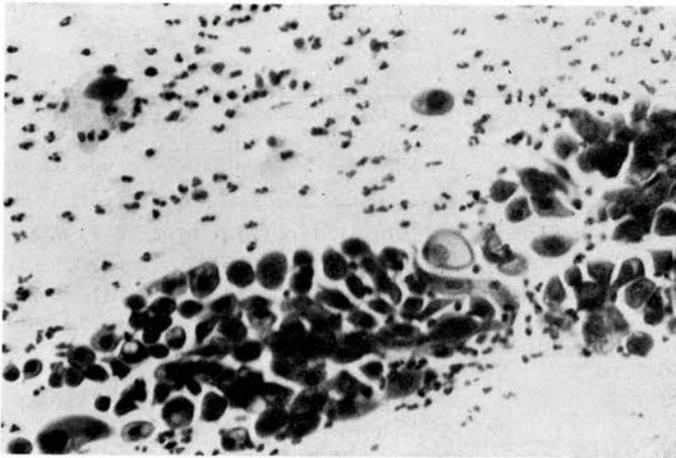


Fig. 4. — Vacuolated metaplastic cells in a patient Chlamydia and *M. Homnis* culture positive (Papanicolaou $\times 250$).

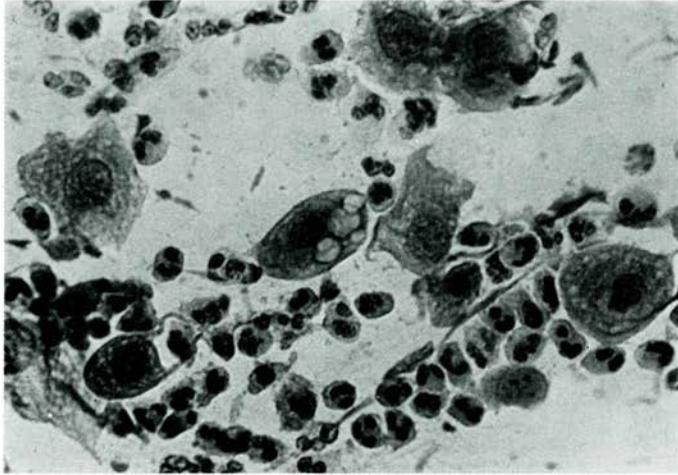


Fig. 5. — Metaplastic cells (with « bubbly cytoplasm ») in a patient Chlamydia, *M. hominis*, *U. Urealyticum* culture positive (Papanicolaou $\times 400$).

koilocytic squamous cell (fig. 5). Three of the 4 cases with CIN I, and 2 of the 3 with CIN III had CT positive cultures confirmed by colposcopy; *M. hominis* was isolated in 2 cases, *U. Urealyticum* in all three (table 2).

We agree with Edwards (7) that vacuolated metaplastic cells and those with "bubbly" cytoplasm are seen in the presence of other infections and are thus not

necessarily an expression of Ct. A cytological diagnosis of Ct can therefore only be made when the smears contain the classical Gupta type-3 inclusion bodies. An exact diagnosis thus requires strict cytological criteria and a comparison of the results of microbiological cultures and direct-immunofluorescence tests or both, cytological studies and colposcopy. No clear etiological link emerged in our spe-

Table 2.

No. smears	Cytological observation	<i>C. trachomatis</i>		<i>M. hominis</i>	<i>U. urealyticum</i>
		Culture positive no.	IF positive no.	Culture positive no.	Culture positive no.
9	Classical chlamydial inclusions	9 (23.07%)	8 (20.51%)	1 (2.56%)	1 (2.56%)
8	Vacuolated metaplastics	3 (7.69%)	1 (2.56%)	4 (10.25%)	2 (5.12%)
6	Bubbly cytoplasm of metaplastics	3 (7.69%)	2 (5.12%)	5 (12.82%)	2 (5.12%)
4	Intravacuolar bodies	0	1 (2.56%)	2 (5.12%)	1 (2.56%)
2	Koilocytic squamous cells	2 (5.12%)	2 (5.12%)	0	0
4	CIN I + HPV	2 (5.12%)	3 (7.69%)	0	1 (2.56%)
3	CIN III	2 (5.12%)	3 (7.69%)	2 (5.12%)	3 (7.69%)
1	CIS	1 (2.56%)	0	0	0
2	CIS treated by conization	0	0	0	0

Total = 39

cimens, between Ct infection and *M. hominis* or *U. urealyticum*, all of which are pathogens frequently found in the vagina (2, 11, 14).

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