Diagnosis of *Mycoplasma hominis* Pyelonephritis by Demonstration of Antibodies in Urine

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To evaluate the diagnostic significance of the demonstration in urine of antibodies to *Mycoplasma hominis*, 1,000 samples of urine with more than 5 leukocytes per high-power field were serologically investigated by indirect hemagglutination, using glutaraldehyde-fixed erythrocytes coated with *M. hominis* antigen. The samples were collected from 702 patients. Antibodies were demonstrated in the urine of nine patients, all of whom had signs of acute attack of pyelonephritis. In seven of these patients, characterized by mild or moderate clinical signs and absence of lower urinary tract symptoms, bacterial causes were not observed, whereas *M. hominis* organisms were isolated from the upper urinary tract in most cases and from the bladder urine in all cases. In two patients, characterized by severe clinical signs and presence of lower urinary tract symptoms, both *M. hominis* and bacteria were isolated from the upper urinary tract and bladder urine. The demonstration of antibodies to *M. hominis* in urine is of high diagnostic value as they were only observed in patients in whom *M. hominis* infection in the upper urinary tract was evident or likely and only in the presence of clinical signs of acute attacks of pyelonephritis.

Mycoplasma hominis has been isolated from the upper urinary tract of patients with acute pyelonephritis (16) and acute exacerbation of chronic pyelonephritis (15), in several cases without a bacterial cause of the disease being demonstrated. *M. hominis* has not been cultivated from the upper urinary tract of patients with noninfectious urinary tract diseases (14, 16). The occurrence of *M. hominis* in the upper urinary tract was frequently followed by a significant serum antibody response and, furthermore, by the appearance of urine antibodies, which has been observed only in cases of upper urinary tract infections by *M. hominis* (17). Thus, it seems reasonable to presume that *M. hominis* may be a cause of acute episodes of pyelonephritis in humans and that the demonstration of antibodies in urine is of diagnostic significance.

The purpose of the present study was to verify whether patients with urine antibodies had *M. hominis* infection in the upper urinary tract and to study the clinical signs and course and the diagnostic possibilities of the infection.

MATERIALS AND METHODS

Patients. A total of 1,000 urine specimens, each containing more than 5 leukocytes per high-power field ($\times 400$ magnification) after centrifugation at 1,500 rpm for 5 min, were examined for antibodies to *M.*

hominis. The specimens originated from 702 patients, viz., 250 men and 452 women whose ages averaged 66 (18 to 82) and 59 (16 to 78) years, respectively. Most of the cases were outpatients received for ordinary examinations, and the diagnosis and point in the course of illness could not be established.

Only patients showing antibodies in the urine were studied further. A thorough clinical examination was performed; several samples of urine from the bladder and, as far as possible, from the upper urinary tract were collected for cultivation of mycoplasma and bacteria, and serum samples were obtained.

The diagnosis of acute pyelonephritis was based on the simultaneous occurrence of acute lumbar pain, elevated body temperature, and more than five leukocytes per high-power field in centrifuged urine, followed by complete recovery.

Acute exacerbation of chronic pyelonephritis was diagnosed by the presence of acute lumbar pain and elevated body temperature in patients with at least three of the following signs of chronic pyelonephritis: repeated attacks of urinary tract infections, pyuria, impaired renal function, and demonstration of chronic interstitial nephritis by histological examination or irregular outline of the kidney associated with dilated calyces by radiography.

Sampling. (i) Bladder urine. Urine from the bladder was obtained before the onset of the attack in three cases (Table 1), and in all cases it was obtained at several times during the attack and at approximately monthly intervals during the 3 months after the attack. The urine was collected as clean-voided, midstream urine or by catheterization.

(ii) **Ureteric urine.** Ureteric urine was collected from seven patients, in all cases during the attack (Table 1). After the introduction of a cystoscope and washing of the bladder with water, urine from the upper urinary tract was collected through bilaterally placed ureteric catheters. The urine was separated into initial, middle, and terminal portions.

(iii) **Serum.** Serum samples were collected in all cases at several times during the attack and at approximately monthly intervals during a 3-month period after the attack.

All specimens were stored at 4°C before examination, which was carried out within 24 h of collection. Storage at room temperature during collection, transportation, and examination did not exceed 2 h. After examination the specimens were stored at -20°C.

Cultivation and identification. All urine specimens from patients showing antibodies to *M. hominis* were cultivated for mycoplasma and bacteria within a few hours after sampling.

Mycoplasma were cultivated on a modified Hayflick medium in candle jars and in 95% N₂ plus 5% CO₂ and identified by the growth inhibition test and indirect immunofluorescence as previously described (15).

Bacteria were cultivated on blood agar, lactose bro-mothymol blue agar, and chocolate agar, in both atmospheric air and 100% H₂. The bacteria were identified according to colony morphology, Gram staining, and biochemical properties.

Demonstration of antibodies to *M. hominis*. Urine specimens were examined within 24 h of sampling for antibodies to *M. hominis* by the use of indirect hemagglutination performed with glutaraldehyde-fixed sheep erythrocytes (1) and pooled antigen (17). The titration was carried out in a microtiter system (5).

(i) **Fixation of erythrocytes with glutaraldehyde.** Sheep erythrocytes collected in Alsever solution were washed four times with phosphate-buffered glucose, pH 7.2, and resuspended to a 20% suspension in phosphate-buffered glucose. Portions of this suspension were mixed with an equal volume of 0.2% glutaraldehyde in phosphate-buffered glucose and incubated at 37°C for 15 min in a water bath. After this fixation, the erythrocytes were washed five times, resuspended to a 10% suspension in normal saline containing 0.1% sodium azide, and stored at 4°C.

(ii) **Production of antigen.** Three strains of *M. hominis*, all isolated from the upper urinary tract of patients with acute pyelonephritis (16), were used as antigen. The strains had different titers when titrated against *M. hominis* (PG21) rabbit hyperimmune serum (17) by indirect immunofluorescence (10). Each strain was cultivated in 600 ml of modified Hayflick medium (2), harvested by centrifugation at 14,000 × *g* for 1 h, and resuspended in 5 ml of normal saline. This suspension contained approximately 10¹⁰ colony-forming units of *M. hominis* per ml and was stored at -20°C.

(iii) **Coating with antigen.** Glutaraldehyde-fixed erythrocytes were washed twice and diluted in phosphate-buffered saline, pH 7.0, to a 20% suspension. Primarily, erythrocytes were coated with each antigen separately by mixing 2 ml of antigen suspension with 5 ml of the 20% erythrocyte suspension. This mixture

was incubated for 18 h at 37°C, washed three times, and resuspended to a 20% suspension in phosphate-buffered saline containing 50% glycerine.

(iv) **Titration and pooling of the antigen.** Erythrocytes coated with twofold dilutions of each antigen were washed three times, resuspended in phosphate-buffered saline to a 2% concentration, and titrated separately by indirect hemagglutination against *M. hominis* (PG21) rabbit hyperimmune serum in the microtiter system as described for fresh erythrocytes (5). Phosphate-buffered saline containing 1% normal rabbit serum was used as diluent. According to the results of these titrations, the three antigens were diluted in normal saline to the optimum concentrations.

Equal volumes of these antigen dilutions were mixed. Glutaraldehyde-fixed erythrocytes were coated with this antigen pool, titrated, and diluted to the optimum concentration of the pooled antigen as described above.

(v) **Titration of specimens.** A batch of glutaraldehyde-fixed erythrocytes coated with the final antigen pool dilution was stored at 4°C and used for the detection of antibodies in specimens from patients during a period of 6 months. Titrations of the samples were performed as described above for antigen.

The stability of glutaraldehyde-fixed *M. hominis*-coated erythrocytes was checked by daily titration of the same hyperimmune serum. The reproducibility of the test was studied by repeated titration of positive specimens at monthly intervals. The specificity was demonstrated by titration of sera containing antibodies to other human mycoplasmas. The sensitivity of the test using glutaraldehyde-fixed and fresh erythrocytes (5) was compared.

RESULTS

Stability, specificity, and sensitivity of glutaraldehyde-fixed, *M. hominis*-coated sheep erythrocytes. Glutaraldehyde-fixed, *M. hominis*-coated erythrocytes were stable for 6 months after production, and during this period the indirect hemagglutination test was reproducible as determined by repeated titration of serum specimens. Urine antibodies were generally not preserved after storage at -20°C and were recovered from only 1 of 12 positive samples by repeated titration.

The specificity and sensitivity of the test were satisfactory. Glutaraldehyde-fixed erythrocytes coated with *M. hominis* did not react with human sera containing antibodies to seven other human *Mycoplasma* species, and they showed titers as high as those of fresh erythrocytes.

Patients with urine antibodies to *M. hominis*. Nine patients, four men and five women, had urine antibody titers of ≥16 to *M. hominis*. All nine had acute attacks of pyelonephritis; six had acute pyelonephritis, and three had acute exacerbation of chronic pyelonephritis. Cases in which bacteria could not be isolated from the upper urinary tract and with less than 10⁴ of a

mixed flora of bacteria per ml in bladder urine during and after the attack were considered not to be caused by bacteria.

Based on clinical signs (pain, tenderness, lower urinary tract symptoms, body temperature, and number of leukocytes in the urine), the nine patients could be divided into three groups (Table 1).

(i) Mild acute attacks of pyelonephritis.

Two patients had tenderness in the costovertebral angle on one side. Pain and lower urinary tract symptoms were absent. The temperatures were 37.9 and 38.0°C. The number of leukocytes in urine was not higher than 30 per high-power field. Bacterial causes were not demonstrated.

In one patient (no. 634), ureteric urine from the affected side contained *M. hominis* and antibodies to *M. hominis*. Ureteric urine from the other side was normal. In bladder urine, *M. hominis* and antibodies were demonstrated constantly during the disease. Serum antibodies were not demonstrated. After treatment with tetracyclines, the symptoms, pyuria, and the yield of *M. hominis* and antibodies disappeared, and relapse was not observed for the next 3 months.

The second patient (no. 611, shown in Fig. 1) was not subjected to ureteric catheterization. In bladder urine, *M. hominis* and antibodies were present constantly during the attack. Serum antibodies were not detected. Without antibiotic treatment, the symptoms, pyuria, and urine an-

tibodies disappeared, but *M. hominis* was still isolated from the urine. After 13 days without symptoms, the patient died from pulmonary embolism. At autopsy, the affected kidney revealed, close to the renal pelvis, a small abscess from which *M. hominis*, but no bacteria, could be isolated. Other parts of the kidney were normal macroscopically, and *M. hominis* was not cultivated from these parts or from the renal pelvis.

(ii) Moderate acute attacks of pyelonephritis. Five patients showed unilateral lumbar pain and tenderness, absence of lower urinary tract symptoms, temperatures of 38.2 to 38.6°C, and 20 to 40 leukocytes per high-power field in the urine. Bacterial causes were not demonstrated.

Ureteric urine obtained from four patients contained, on the affected side, *M. hominis* in three cases (no. 10, 136, and 683) and antibodies in all cases. One patient (no. 154) was not subjected to ureteric catheterization. Bladder urine from one patient (no. 136), examined before the appearance of symptoms, did not contain antibodies to *M. hominis* (Fig. 2). During the attack, *M. hominis* and antibodies were demonstrated in bladder urine of all five patients; in two cases (no. 10 and 683), the antibodies were not observed constantly (no. 10, shown in Fig. 2). A significant rise in serum antibodies was demonstrated in four patients (no. 136, [Fig. 2], 154, 683, and 701), whereas in one patient (no. 10) antibodies, although present, did not change sig-

TABLE 1. Clinical characteristics, occurrence of *M. hominis* in urine, and occurrence of antibodies to *M. hominis* in urine and serum in nine patients with acute attacks of pyelonephritis

Group	Patient no.	Clinical characteristics					Occurrence of <i>M. hominis</i>			Antibodies to <i>M. hominis</i> (highest titer)				
		Lumbar pain	Tenderness	Lower urinary symptoms	Body temp (°C)	Leukocytes in urine ^b	Bladder urine	Ureteric urine		Urine ^a			Serum	
								Right side	Left side	Before	During	After	Acute phase	Convalescent phase
I	634	—	+	—	37.9	30	+	+	—	NT ^c	32	<2	<2	<2
	611	—	+	—	38.0	20	+	+	NT	NT	512	<2	<2	<2
II	10	+	+	—	38.4	40	+	—	+	NT	1,024	4	128	256
	136	+	+	—	38.2	20	+	—	+	<2	128	<2	<2	512
	683	+	+	—	38.2	40	+	+	—	NT	128	4	32	256
	701	+	+	—	38.6	40	+	—	—	NT	128	<2	<2	128
	154	+	+	—	38.5	20	+	NT	NT	NT	256	2	64	512
III	251	+	+	+	39.3	200	+	+	—	<2	64	NT	16	256
	414*	+	+	+	39.8	300	+	—	+	<2	64	2	32	512

^a In relation to the attack.

^b Number per high-power field in centrifuged urine.

^c NT, Not tested.

^d Cultivated from the kidney at autopsy.

* This patient had two attacks (see Fig. 4).

FIG. 1. A patient (no. 611) with a mild acute attack of pyelonephritis. Symbols: Occurrence of symptoms (×××××); leukocytes in centrifuged urine per high-power field (vertical bars); *M. hominis*, colony-forming units per milliliter of urine (●); *M. hominis* antibody titers in urine (◆) and serum (▲).

FIG. 2. Two patients (no. 10 and 136) with moderate acute attacks of pyelonephritis. Symbols as in Fig. 1.

nificantly. In two cases (no. 136 and 683), urine antibodies were demonstrated before serum antibodies (no. 136, shown in Fig. 2). After treatment with tetracyclines, symptoms, pyuria, *M. hominis*, and urine antibodies disappeared in all five cases, and relapse did not occur in the following 3 months (no. 10 and 136, shown in Fig. 2).

(iii) **Severe acute attacks of pyelonephritis.** Two patients suffered from intense unilateral lumbar pain, tenderness, and lower urinary tract symptoms, and temperatures of 39.3 and 39.8°C and 200 to 300 leukocytes per high-power field in the urine were recorded. Mixed infections with *M. hominis* and bacteria were demonstrated.

In one patient (no. 251, shown in Fig. 3), ureteric urine from the affected side yielded *M. hominis* and antibodies. Bladder urine examined before the onset of the disease did not harbor antibodies, but urine sampled during the attack contained *M. hominis* and $>10^5$ *Klebsiella pneumoniae* per ml, and antibodies to *M. hominis* occurred periodically. A significant serum antibody response to *M. hominis* was demonstrated. After treatment with ampicillin, the patient improved, but symptoms were still present; the number of leukocytes in the urine decreased to 20 per high-power field. *K. pneumoniae* could not be detected, but *M. hominis* and antibodies were still present in the urine. At this time, the examinations were stopped as the patient suffered from cancer of the colon in the terminal stage.

The second patient (no. 414, shown in Fig. 4) had two acute episodes of pyelonephritis during a 2-month interval. During the first episode, a bacterial cause could not be demonstrated. Ureteric catheterization was not performed, but *M.*

hominis and antibodies were demonstrated in the bladder urine, which contained 25 leukocytes per high-power field. A significant serum antibody response to *M. hominis* developed. This episode was not treated, but symptoms, pyuria, and antibodies disappeared; however, *M. hominis* was still present in the urine. Two months later, the patient had a second attack, this time the attack was accompanied by severe symptoms. In ureteric urine from the affected side and in bladder urine, *M. hominis*, antibodies, and *Escherichia coli* were recovered. After treatment with tetracyclines, the symptoms disappeared, and the urine returned to normal.

DISCUSSION

The results of the present and previous studies on cultivation (15, 16) and antibody demonstration (17) indicate that *M. hominis* is a cause of some acute episodes of pyelonephritis.

In the present study, urine antibodies to *M. hominis* were demonstrated in nine patients, all

FIG. 3. A patient (no. 251) with a severe acute attack of pyelonephritis. Symbols as in Fig. 1.

FIG. 4. A patient (no. 414) with a moderate and a severe acute exacerbation of chronic pyelonephritis. Symbols as in Fig. 1.

of whom showed signs of acute attacks of pyelonephritis. In seven of the patients, a bacterial cause of the disease was not observed, whereas *M. hominis* and antibodies to *M. hominis* were demonstrated in the upper urinary tract of five and in bladder urine of all seven cases. Furthermore, in four of these patients a significant serum antibody response to *M. hominis* developed.

In the remaining two patients, a mixed infection of *M. hominis* and bacteria occurred in the upper urinary tract, and antibodies to *M. hominis* were demonstrated in ureteric urine. In one of these cases, antibiotic eradication of the bacteria was followed by improvement, but not disappearance, of the clinical signs. The other patient had two attacks, the first of which seemed to be caused by *M. hominis* and was cured spontaneously but was followed by continuous yielding of *M. hominis* in the urine. During the second attack, a mixed infection of *M. hominis* and bacteria was demonstrated. Whether an interaction between the microorganisms occurred is not known, but it has been demonstrated that rats experimentally colonized in the upper urinary tract with *Mycoplasma arthritidis* were more susceptible to *E. coli* infection of the kidney (18).

In some cases of pure *M. hominis* infections, the clinical signs were mild and serum antibody responses were not elicited, and in other cases the clinical signs were moderate and regularly followed by serum antibody responses. In experimental *M. arthritidis* pyelonephritis (11), it has been demonstrated that rats with lesions limited to the renal pelvis and associated areas produced only urine antibodies, whereas rats with more extensive lesions produced both urine and serum antibodies. In experimental upper urinary tract infection caused by *E. coli* (3), it was observed that rats with pyelitis did not develop serum antibodies, whereas rats with pyelonephritis did.

All patients with pure *M. hominis* infections were characterized by absence of lower urinary tract symptoms, which is in accordance with the common opinion that *M. hominis* does not cause urethritis or cystitis. In contrast to pure *M. hominis* infections, mixed infections including bacteria were followed by severe clinical signs as well as lower urinary tract symptoms.

Without treatment, the course of *M. hominis* infections seems to be self-limiting. However, the two untreated patients continued to harbor *M. hominis* in the urinary tract, in one case in the kidney. In the other case a relapse with mixed infection of *M. hominis* and bacteria in the upper urinary tract occurred. In patients with bacterial pyelonephritis who receive no or insufficient treatment the bacteria may remain in the kidney and cause relapse (19).

After treatment with tetracyclines, cessation of symptoms and disappearance of *M. hominis* and antibodies occurred within 2 or 3 days in all of the seven treated patients, and for a 3-month period no relapse was observed.

For diagnostic purposes, it has previously been established that *M. hominis* may be cultivated from bladder urine without being present in the upper urinary tract (16). Cultivation from ureteric urine revealing quantitative equal growth of *M. hominis* in three subsequently collected samples indicates infection, but the procedure is laborious.

The demonstration of serum antibodies is of value in those cases in which a significant response occurs, but, as shown in the present study, this usually did not develop until 2 weeks after the onset of disease. Furthermore, it was demonstrated in the present and in previous studies (17) that patients may have *M. hominis* infection of the upper urinary tract not associated with a serum antibody response, and it has also been established (17) that patients may develop serum antibodies to *M. hominis* during acute pyelonephritis without *M. hominis* being isolated from the urinary tract at all.

The demonstration of urine antibodies seems to be of great value. All patients showing urine antibodies to *M. hominis* had signs of acute episodes of pyelonephritis and one or more signs indicating infection of the upper urinary tract by *M. hominis* alone or combined with bacteria. Urine antibodies seem only to be present during infections associated with symptoms. In cases where the urine was examined before the onset of the disease (no. 136, 251, and 414), antibodies could not be demonstrated, and the urine antibodies disappeared with the clinical signs of disease. During an attack, the presence of urine antibodies may fluctuate and periodically disappear (no. 10 and 251). This fluctuation may possibly be due to opening and closing of lesions or to blocking of the antibodies by mycoplasmas.

The urine antibodies were not stable when stored at -20°C . The influence of temperature, pH, and content of albumin on the stability of these antibodies have to be studied.

In a previous study (17), urine antibodies were demonstrated in only 4 of 10 patients with acute episodes of pyelonephritis and *M. hominis* infection in the upper urinary tract. However, only one sample of urine from each patient was examined, and the specimens had been stored at -20°C . Thus, it has not been established whether *M. hominis* infection of the upper urinary tract may be present without shedding of urine antibodies, but the results of the present study emphasize the need for immediate examination of the urine and the need in some cases

for collection of several urine samples during the attack in order to demonstrate antibodies.

The antibody level of diagnostic significance is difficult to establish even when the examinations are performed with one reproducible method. In this study, the demonstrated levels varied greatly, and they were not correlated to the severity of the infection (Table 1). However, a urine antibody titer of ≥ 32 in one or more samples was demonstrated to imply *M. hominis* infection of the upper urinary tract.

The urine antibodies seem to originate from the kidneys. They were detected in ureteric urine, but only from the infected side, and in several cases they were present in urine but not in simultaneously collected serum. They occurred only during the acute attack and preceded the development of serum antibodies. This is in accordance with the observations of experimental *M. arthritidis* pyelonephritis (11). In bacterial pyelonephritis, urine antibodies occur both in natural (20) and in experimental infections (4, 7). Under the experimental conditions, they seemed to originate from the kidneys and at least in some cases to be secretory immunoglobulin A antibodies (12). Moreover, the occurrence in urine of bacteria coated with antibodies is supposed to imply an upper urinary tract infection (13).

For serological diagnosis of *M. hominis* infection, some recent studies (8) have indicated that indirect hemagglutination is more reliable than the tests for metabolism inhibition, immunofluorescence, and growth inhibition. The use of glutaraldehyde-fixed sheep erythrocytes is recommended for indirect hemagglutination testing. Antigen-coated erythrocytes were stable for 6 months, and reproducible results were obtained during this period. The specificity and sensitivity were satisfactory, as has also been shown in other studies on *M. hominis* (6), *M. pneumoniae* (9), and bovine mycoplasmas (1).

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