JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1982, p. 487–489
0095-1137/82/090487-03$02.00/0

Vol. 16, No. 3

Rapid Evaluation of Female Patients Exposed to Gonorrhea by Use of the *Limulus* Lysate Test

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Received 16 April 1982/Accepted 28 May 1982

The *Limulus* amoebocyte lysate (LAL) assay was used to evaluate 115 females who were named as sexual contacts by men with culture-proven gonorrhea. These patients were treated for gonorrhea before laboratory confirmation, as recommended by the Centers for Disease Control, because of the lack of rapid screening tests and the serious consequences of undetected infection. For the LAL assay, endocervical samples were collected with depyrogenated cotton-tipped swabs, and the swabs were placed in 10 ml of diluent to assay for endotoxin; the negative predictive value of the LAL assay at this dilution was 100%. Incubation was carried out at 37°C for 30 min; positive or negative results were indicated by gelation or lack of gelation, respectively. Lysate sensitivity was 0.3 ng/ml, with an *Escherichia coli* endotoxin standard. Single endocervical cultures and the LAL assay were both positive in 71 patients, but the Gram stain was positive in only 36 (50.7%) of these cases. For the 44 culture-negative cases, the LAL assay was negative in 21 (47.7%). Thus, the LAL assay was able to selectively exclude approximately half of the culture-negative gonorrhea contacts and would have spared these patients inappropriate therapy and contact tracing, without excluding culture-positive gonorrhea cases.

The Centers for Disease Control currently recommend that sexual contacts of patients with culture-proven gonorrhea be treated for gonorrhea before the presence or absence of disease is confirmed by culturing (3). This indiscriminate approach to antibiotic therapy is prompted by the lack of rapid screening tests and the serious consequences of untreated gonorrhea. The Gram stain is the only accepted procedure in clinical medicine that is used for an immediate diagnosis of gonococcal cervicitis (13). However, the Gram stain requires the expertise of trained microscopists and has a low sensitivity, ranging from 50 to 65% (1, 2, 4). Therefore, information derived from a negative Gram stain is meaningless in attempting to exclude the possibility of gonococcal cervicitis.

Recently, we reported our experience with the *Limulus* amoebocyte lysate (LAL) assay in evaluating women with gonococcal cervicitis (10). Whereas the predictive values of a positive test ranged from 36.5 to 97.4% for prevalence rates of 1 to 40%, respectively, the predictive value of a negative test was 100%. Thus, a negative LAL assay would exclude the possibility of gonococcal cervicitis. We report here the results of our using the LAL assay to evaluate female patients exposed to gonorrhea and the ability of the assay to exclude those patients without gonococcal cervicitis.

(This work was presented in part at the 82nd Annual Meeting of the American Society for Microbiology, Atlanta, Ga., 7 to 12 March 1982 [R. B. Prior and V. A. Spagna, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C18, p. 274].)

MATERIALS AND METHODS

Patient population. A group of 115 women who were named as sexual contacts by men with culture-confirmed gonorrhea and who attended the Columbus Health Department Venereal Disease Clinic for treatment comprised the study group. The mean age of the patients was 22.7 years, with a range from 18 to 34 years.

Patients were excluded from the study if they (i) were under 18 years of age or menopausal, (ii) were pregnant, (iii) lacked a cervix or had surgical injury to the cervix, (iv) were menstruating at the time of examination, (v) admitted to antibiotic usage within 10 days of the examination, or (vi) failed to give informed written consent.

Diagnostic procedures. Each patient underwent a standardized interview concerning demography and sexual and venereal disease history, an examination of the genitalia and inguinal lymph nodes, and a pelvic examination which included the use of a speculum to visualize the cervix. Endocervical samples were collected as previously described (10). Briefly, the ecto-

TABLE 1. Results of Gram-stained smears and LAL assays for 115 female patients exposed to men with confirmed gonorrhea

Culture result[a]	No. of patients	Gram-stained smears		LAL assays	
		No. positive	No. negative	No. positive	No. negative
Positive	71	36	35	71	0
Negative	44	0	44	23	21

[a] Results were obtained from a single endocervical culture obtained before therapy. Positive, *N. gonorrhoeae* isolated; negative, *N. gonorrhoeae* not isolated.

cervical mucus was first removed with a pyrogen-free foam sponge, and then a depyrogenated cotton-tipped swab was inserted approximately 1.5 cm into the endocervical canal for 10 s. Care was taken not to touch the vaginal vault with the swab during the collection procedure. The entire swab was then placed into a plastic test tube (no. 2045; Falcon Plastics, Oxnard, Calif.) containing 10 ml of an aqueous 5% metallo-modified polyanionic dispersing agent (Pyrosperse; Mallinckrodt, Inc., St. Louis, Mo.). The dispersing agent and 10 ml of diluent were used because a sensitivity of 100% was obtained with this diluent and volume (10).

For cultures, sterile cotton-tipped swabs were inserted approximately 1 cm into the endocervical canal and then streaked directly onto Thayer-Martin medium for isolation of *Neisseria gonorrhoeae*. The plates were incubated at 35°C in 5% CO_2 for 48 h. Cultures for viruses or chlamydiae were not done.

The same procedure was used to obtain endocervical samples for Gram staining and subsequent microscopic examination.

Laboratory methods. Gram-stained smears of endocervical exudate were examined under 1,000× magnification oil immersion for gram-negative diplococci and polymorphonuclear cells. Smears were considered positive only if typical gram-negative diplococci were seen to be located intracellularly in the polymorphonuclear cells. All isolates grown on Thayer-Martin medium were confirmed as *N. gonorrhoeae* by typical sugar fermentation reactions.

***Limulus* lysate assay.** The endocervical samples were assayed for the presence of endotoxin by the LAL assay. Tubes containing the swabs were mixed on a Vortex mixer, and 0.25 ml from each tube was transferred to single-test lysate vials (Mallinckrodt). The lysate vials were gently mixed, incubated undisturbed for 30 min at 37°C in a heating block, and read. A firm gel that remained adherent to the bottom of the lysate vial when the vial was carefully inverted 180° was interpreted as a positive test; the absence of firm gelation was interpreted as a negative test. The minimum sensitivity of the LAL assay was the detection of 0.3 ng of *E. coli* endotoxin (lot EC-2; Bureau of Biologics, U.S. Food and Drug Administration, Bethesda, Md.) per ml.

Analysis of data. Results of the LAL assays, Gram-stained smears, and cultures were entered into a Hewlett-Packard programmable computer (model 9825A) for subsequent determinations of correlation. Sensitivity, specificity, and predictive values were computed by using the methods described by Vecchio (12).

RESULTS

The results of Gram-stained smears and the LAL assays for 115 female patients exposed to men with confirmed gonorrhea are shown in Table 1. Single endocervical cultures were positive for *N. gonorrhoeae* in 71 (61.7%) of these cases and negative in 44 (38.3%). The Gram stain was positive in 36 (50.7%) of the 71 culture-positive cases and universally negative in the culture-negative cases. The negative predictive value of the Gram stain was 55.7%. The LAL assay was positive in all 71 (100%) culture-positive cases and negative in 21 (47.7%) of the 44 culture-negative cases. The negative predictive value of the LAL assay was 100%.

DISCUSSION

Epidemiological treatment of gonorrhea refers to the use of antibiotic therapy when a diagnosis of gonorrhea is considered likely but before confirmatory culture results are obtained (6). The Centers for Disease Control currently recommend treatment for men and women known to have been recently exposed to gonorrhea, and the treatment regimens recommended are the same as those for laboratory-confirmed uncomplicated gonorrhea (3). This recommendation to treat all exposed persons is warranted for several reasons. First, the prevalence of gonorrhea in women exposed to the disease is high. In a recent study of female contacts of men with gonorrhea, 66% were found to be positive for gonorrhea (1). In our study, the frequency of gonorrhea confirmed by a single endocervical culture was 61.7%. Second, rapid methods to evaluate female patients at the time of their presentation are lacking, and clinical judgment alone is insufficient to make a diagnosis, since a majority of women with gonococcal cervicitis have no signs or symptoms or both (8, 10). Third, serious complications can occur in patients with undetected and untreated gonorrhea. In the United States it is estimated that over 200,000 cases of gonococcal pelvic inflammatory disease occur annually, and this disease is a relatively early complication of gonorrhea (5).

However, with the epidemiological treatment approach, all individuals not infected also receive therapy, and it is estimated they constitute 21 to 47% of those seeking treatment because of exposure (6). Treatment of the uninfected (i) needlessly exposes patients to adverse effects of antibiotics, (ii) is an unjustified expense, (iii) raises a dilemma as to the management of sexual partners, (iv) fosters return visits by many patients for therapy to relieve their anxieties, and

(v) subjects many patients to the psychosocial discomfort of being treated for a venereal disease (6, 7). Additionally, epidemiological treatment may promote selection of antibiotic-resistant bacteria (6). In the study reported here, 38.3% of the contacts were uninfected as determined by culturing and received unnecessary treatment. This figure, however, may not represent the true number of uninfected cases, since a single cervical culture only detects gonococcal cervicitis in about 80 to 90% of patients (2, 11, 14).

Excluding the possibility of a sexually transmitted disease and assuring patients that they are not infected are at least as important as making the proper diagnosis for infected patients (7). The accuracy of the Gram stain in quickly eliminating patients without gonococcal cervicitis was poor, with a negative predictive value of 55.7%. Thus, 44.3% of patients with negative Gram stains could have gonococcal cervicitis. Recently, the LAL assay was shown to be a rapid method for the evaluation of females with gonococcal cervicitis (9, 15), and when endocervical samples were collected with swabs and properly diluted, the predictive value of a negative test to exclude patients without gonococcal cervicitis was 100% (10). Thus, a negative LAL assay correlated highly with the absence of cervical gonorrhea. In the study reported here, the LAL assay and single endocervical culture were both positive in 71 gonorrhea patients. For the 44 culture-negative cases, the LAL assay was negative in 21 (47.7%). Therefore, the LAL assay was able to exclude selectively approximately half of culture-negative gonorrhea contacts and would have spared these patients inappropriate therapy and contact tracing, without excluding culture-positive gonorrhea cases. Although the reasons for the 23 false-positive LAL assay results are speculative, culture failures, unreported antibiotic usage, vancomycin-sensitive strains, and contaminated (pyrogen-containing) equipment may provide an explanation. Moreover, false-positive reactions could occur due to contamination with endotoxin from the vaginal flora, since the LAL assay is not a specific test for *N. gonorrhoeae*.

The LAL assay was easy to perform, and test results were available within 30 min of sample collection. The overall simplicity of the test would allow for its use outside of sophisticated medical laboratories by personnel who may be unskilled in complex laboratory procedures. This would allow for large-scale applications and would provide helpful information to both physicians and patients.

ACKNOWLEDGMENTS

We thank Joseph G. Lossick, Medical Director of the Columbus Health Department Venereal Disease Clinic, and his staff for their cooperation and assistance.

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