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Comparison of Three Serological Tests in Gonococcal Infection

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Three serological tests used in the diagnosis of gonococcal infection were compared with cultural techniques in 857 females attending the Prenatal and Gynaecology Clinics at the Winnipeg General Hospital. The tests evaluated were the microflocculation technique (MFT), the indirect fluorescent-antibody technique (IFAT), and the complement-fixation technique (CFT). One hundred six patients had positive cultures for *Neisseria gonorrhoeae*. In this population, the MFT was reactive in 80 patients (75.4%), the IFAT was reactive in 74 (69.8%), and the CFT was reactive in 33 (31.1%). In the 751 patients with negative cultures, the MFT was positive in 11.4%, the IFAT was positive in 17.4%, and the CFT was positive in 10.5%. Sera from 9 of 10 patients with gonococcal arthritis were positive with the MFT.

Gonococcal disease is currently epidemic (1). This has occurred in spite of our knowledge of the epidemiology of the disease, efficient therapeutic agents, and effective cultural diagnostic methods. Perhaps the single most important factor contributing to this failure is the large reservoir of untreated asymptomatic females with gonococcal infections. The control of gonorrhea, therefore, may depend upon the detection of subclinical infection in this group of persons. To identify more of these infections, a simple, sensitive, specific serological test is needed. This study is concerned with such a test and compares it with other serological tests in the diagnosis of gonococcal infection.

MATERIALS AND METHODS

Sera examined. Sera (996) were tested by three serological techniques: microflocculation (MFT), indirect fluorescent antibody (IFAT), and complement fixation (CFT). Eight hundred fifty-seven sera were from patients who had cervical cultures for the diagnosis of gonococcal infection in the Prenatal and Gynaecology Clinics at the Winnipeg General Hospital. Most of these patients were being screened routinely on admission to the clinic; a few were gonococcal contacts or had symptomatic infection. Additional sera were obtained from 10 patients with gonococcal arthritis, 21 healthy laboratory staff members, 41 children presumed not to be infected with *Neisseria gonorrheae*, 53 meningococcal carriers in an Eskimo population (39 with group B, 7 with group Y, and 7 with ungroupable strains), 10 patients with group A meningococcal disease, and 4 adults who were nasopharyngeal carriers of *N. lactamicus*. The sera were stored at −20 C until tested.

Bacterial cultures. The specimens for culture were obtained with a swab and immediately streaked on Thayer-Martin medium (5).

Antigens and antisera. Three different antigen preparations were used for the three serological procedures. The MFT as described by Reising (4) uses an antigen suspension consisting of cholesterol lecithin particles sensitized with a sonically treated extract of gonococci. To prepare the extract, gonococci (strain F62 type 1) cultivated on G.C. medium base with a defined supplement (2) were scraped with a bent-glass rod into sterile distilled water, cooled in ice, and then sonicated with the Raytheon 10-kc Magnetostrictive oscillator at 250 W for 1 h. The sonicate was then centrifuged at 43,500 × *g* for 1 h, and the supernatant was decanted and lyophilized. The antigen for the IFAT was prepared by growing strain F62 on a gonococcal base medium. Colonies of type 1 morphology were selected and harvested in distilled water. After dispersion with a Vortex mixer, the suspension was adjusted to 50% transmission in a Beckman spectrophotometer. A drop was placed on the slide and dried. We attempted to have a density of 100 to 200 organisms per high-power field. The CFT (6; as modified by the Ontario Ministry of Health Laboratory) used physically disrupted gonococcal organisms. The bacterial suspension was passed through a Ribi cell fractionator at 15,000 psi. The final preparation contained both protoplasmic and cell wall constituents.

Test procedures. The MFT was carried out as described by Reising (4). Briefly, 0.025 ml of unheated, undiluted serum was pipetted into paraffin-ringed glass slides. Then 0.025 ml of test antigen suspension was added to each serum. The test was only performed with undiluted sera. The slide was then rotated on a Thomas rotating apparatus at 140 rpm for 10 min. An AO series 58 stereoscopic micro-

scope at a ×20 magnification was used to detect agglutination. Known positive sera were used as controls with each batch of sera, and the tests were carried out with groups of 20 sera.

The IFAT was carried out as described by Welch and O'Reilly (7). A 0.05-ml amount of a 1:16 dilution of the serum in phosphate-buffered saline (pH 7.2) was added to the antigen; the mixture was then incubated in a moist chamber for 0.5 h at 37 C. By using distilled water adjusted to pH 9.0 with 1 N NaOH, slides were rinsed and soaked for 5 min. A 1:200 dilution of conjugated antihuman gammaglobulin was added to each antigen spot and incubated. Slides were then rinsed, soaked for 5 min in distilled water, and dried with air pressure. The slides were then read by using a Leitz fluorescent microscope with an Osram HBO-200 mercury lamp a Schott BG-12 exciter filter and an OG-1 barrier filter. Control slides were prepared by using saline in place of the patient's serum as well as slides prepared with known negative and positive sera. A positive result consisted of individual separate cells which fluoresced brightly; a negative result consisted of minimal or no fluorescence.

The CFT was performed as described by Public Health Service Monograph no. 74, 1965 (6).

RESULTS

Eight hundred fifty-seven patients were studied from May 1970 to June 1971 with the three serological tests as well as by culture. Of the 106 patients from whom *N. gonorrhoeae* was isolated, the sera of 80 (75.4%) were reactive by the MFT, 74 (69.8%) were reactive with the IFAT, and 33 (31.1%) were reactive with the CFT. Of the 751 patients from whom *N. gonorrhoeae* was not isolated, the sera of 86 (11.4%) were reactive with the MFT, 131 (17.4%) were reactive with the IFAT, and 79 (10.5%) were reactive with the CFT. (Table 1)

Table 2 reviews the false positives obtained by the three serological techniques. All patients with positive serology were further investigated. A search was made through the files of the Manitoba Department of Health, Venereal Disease Clinic, for a history of recent gonococcal infection, and further specimens were obtained for culture. The historical investigation revealed that 11 of the 86 patients with a false-positive MFT had a recently proven gonococcal infection. An additional 10 patients were positive on repeat culture, suggesting that the initial culture may have been a false negative. Three patients had a positive VDRL. Sixty-two of the 86 patients had no history of venereal disease. Only 9 of the 131 false positives with the IFAT and 10 of the 66 false positives with the CFT had either a recent history of gonococcal infections or were positive on the second culture.

To compare the specificity of these three serological procedures, 62 sera from persons with no historical evidence of a gonococcal infection were tested (Table 3). Of 21 healthy laboratory staff, the MFT and IFAT were negative in all and the CFT was positive in 1. Of 41 children hospitalized for a variety of reasons, 1 was positive with the MFT, 9 were positive with IFAT, and 7 were positive with CFT.

To further study the specificity of the MFT, the sera from 53 persons with positive nasopharyngeal cultures for *N. meningitidis* were tested (Table 4). Twenty-five were children and 28 were adults. Of the 25 children, all were negative with the MFT, 3 were positive with the IFAT, and 4 were positive with the CFT. Of the 28 adults, 4 were positive with all 3 tests. No cultures were done on these persons for *N. gonorrhoeae*.

Sera from four nasopharyngeal carriers of *N. lactamicus* were similarly investigated. No reactivity was noted in the MFT or CFT. One serum was reactive with the IFAT.

Table 5 compares the serological tests in acute and convalescent sera of patients with group A meningococcal disease. The *N. meningitidis* hemagglutinating titers are shown. High titers of meningococcal antibody were not associated with a positive MFT, and there was no conversion of the MFT with rising titers to *N. meningitidis*. Two adults with meningococcal infection had positive titers in all three tests; gonococcal infection was not excluded.

Table 6 compares the three serological techniques in 10 patients with a presumptive diagnosis of gonococcal arthritis. The diagnosis was based on a typical clinical presentation and in nine instances, on a positive culture for *N. gonorrhoeae*. In two patients, joint cultures were positive for *N. gonorrhoeae;* in the other pa-

TABLE 1. *Three serological techniques in females screened for gonococcal infection*

Source of sera	No. tested	MFT		IFAT		CFT	
		No. reactive	%	No. reactive	%	No. reactive	%
Infected female	106	80	75.4	74	69.8	33	31.1
Presumed noninfected female	751	86	11.4	131	17.4	79	10.5

TABLE 2. *Further investigation of serological false positives with negative initial cultures*

Determination	MFT[a]	IFAT	CFT
Recent history of gonococcal infection	11	5	3
Repeat culture positive for *N. gonorrhoeae*	10	4	7
VDRL positive	3	3	3
Past history of venereal disease	62	119	66

[a] Number of false positives: MFT, 86; IFAT, 131; and CFT, 79.

TABLE 3. *Comparison of serological tests in "normal" uninfected controls*

Source of sera	No. reactive		
	MFT	IFAT	CFT
Laboratory Personnel (21)	0	0	1
Children (41)	1	9	7

TABLE 4. *Comparison of serological tests in meningococcal carriers*

Source of sera	No. reactive		
	MFT	IFAT	CFT
Children (25) (5–13 years)	0	3	4
Adults (28)	4	6	4

tients a positive culture originated from a urethral or cervical swab. Patient number 4 was treated prior to culture. Nine of the 10 patients had a positive MFT at the time of initial diagnosis. Six patients had a positive IFAT, and seven patients had a positive CFT.

DISCUSSION

Serological tests are potentially useful in gonococcal disease as an epidemiologic rather than a diagnostic tool. For the investigation of the individual patient, cultural techniques using selective media, such as Thayer-Martin, are inexpensive and specific. A serological procedure could be useful in screening populations to identify asymptomatic carriers of infection. Sero-reactive persons could then be definitively diagnosed by culture and treated.

Since the early 1900s, investigators have looked for the ideal serological test to diagnose gonococcal infection. Unfortunately, a lack of both sensitivity and specificity of tests have frustrated that goal. Recently, the Venereal Disease Research Laboratory, Center for Disease Control, Atlanta, Ga., has identified several antigens from the gonococcus that may be important in the immune response. One of these, the soluble antigen used in the MFT, presumably originates from gonococcal protoplasm and not from cell wall. Flocculation of cholesterol-lecithin particles sensitized with this antigen by specific serum antibodies has proven, in our experience, to be a simple technique to detect gonococcal infection in most women.

A previous study with this antigen on 262 females with gonococcal infection demonstrated 79% to be reactive (4). We found the MFT positive in 75.4% of 106 infected females. Although the initial false positive rate was unacceptably high (11.4%), further documentation of these patients disclosed recent or missed gonococcal infection in 2.8%. In fact, 10 patients with gonococcal infection were only detected

TABLE 5. *Comparison of serologic tests in acute meningococcal disease*

Age/sex	*N. meningitides*	Hemagglutination titer	MFT	IFAT	CFT
1/F	A[a]	8	–	–	–
	C	8	–	–	–
44/M	A	64	+	+	+
	C	32	+	+	+
7/M	A	32	–	–	–
	C	128	–	–	–
16/M	A	256	+	+	+
	C	1,024	+	+	+
63/M	A	128	–	+	+
	C	128	–	+	+
45/M	A	256	–	–	–
	C	128	–	–	–
3/F	A	512	–	–	+
7/M	A	<2	–	+	–
	C	32	–	+	–
4/M	A	128	–	+	–
1/F	A	16	–	–	–

[a] A, Acute; C, convalescent.

TABLE 6. *Comparison of serological tests in gonococcal arthritis*

Sex	Culture	MFT	IFAT	CFT
M	Positive	+	+	1:32
F	Positive	+	+	—
M	Positive	+	+	1:16
M	Negative	+	–	1:32
F	Positive	–	–	–
F	Positive	+	–	–
M	Positive	+	+	1:8
F	Positive	+	+	1:16
F	Positive	+	–	1:16
M	Positive	+	+	1:64

because of concurrent screening with the MFT. The MFT compares favorably in its sensitivity with both the IFAT and CFT as performed in our laboratory. In this series, no additional information was obtained by doing the IFAT and the CFT.

The specificity of the MFT was compared with the IFAT and CFT in a group of normal healthy adults and children and in a group of patients infected with meningococci. An earlier study had demonstrated the IFAT to be ineffective in differentiating meningococcal from gonococcal antibody (3). In our experience, the MFT was moderately specific for gonococcal infection with only one positive result in 62 uninfected normals and few false positives in either symptomatic or asymptomatic meningococcal infection.

Serology is an important tool in the diagnosis of gonococcal arthritis. Frequently, the organism is not recovered either from joint fluid or blood. Most patients with systemic gonococcal infection have antibodies, and in our experience the MFT was a useful diagnostic asset. It again compared favorably with both the IFAT and the CFT.

The MFT technically is a simple test. The antigen remains stable in its lypholized state for a period of at least 18 months. The equipment for the test is present in most diagnostic laboratories. Further investigations are needed to determine the time of appearance of the antibody in relation to infection, the length of persistence of the antibody, and the specific type of immunoglobulin response. This information will give insight into the limitations of the procedure and may suggest further improvements.

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