

STUDIES ON THE INFLUENCE OF ANTIGENS ON THE RESULTS WITH THE GONOCOCCAL COMPLEMENT FIXATION TEST IN PATIENTS WITH UNCOMPLICATED AND COMPLICATED GONORRHOEA

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Abstract. The gonococcal complement fixation test (GCFT) was investigated with regard to its sensitivity and specificity by testing serum specimens from (a) female patients attending a VD out-patient clinic because of suspected gonorrhoea, (b) patients with a proven, uncomplicated, urogenital gonococcal infection, (c) patients with disseminated gonococcal infection (DGI). Three different pools of gonococcal (GC) antigens were used which were comprised of GC strains from two different geographical areas. It was found that 39% of the females with culture-proven uncomplicated gonorrhoea had a positive GCFT whereas 10% of the females with negative GC cultures had a positive GCFT. The latter were found to have either a history of gonorrhoea or strong clinical suspicion of recent GC infection. One of the GC antigen pools gave a much lower diagnostic yield than the other two pools in the GCFTs with serum specimens from patients with uncomplicated gonorrhoea. However, no differences were found between the antigen pools in the tests with serum specimens from patients with DGI. These findings indicate the presence of various strain antigens participating in the immune response to complicated as well as uncomplicated GC infections. The results are presented in detail and discussed.

Key words: *Neisseria gonorrhoeae*; Complement fixation test; Serology; Immunology; Antigens

Gonococcal serology for diagnostic purposes dates back to the beginning of this century and has enjoyed a continued interest ever since. Many different tests such as complement fixation, haemagglutination, precipitation, agar gel diffusion and radioimmune assays have been proposed and used by many authors. The antigen preparations used in these tests have been derived from avirulent or virulent gonococcal strains with no or very scanty information as to the characteristics of their anti-

genic set-up, mainly due to the fact that we still do not have an accepted serotyping system for *Neisseria gonorrhoeae*. The literature and relevant works on these subjects were recently reviewed by Holmes (5) and by Sandström & Danielsson (14).

None of the proposed serological tests has yet gained general acceptance. However, the gonococcal complement fixation test (GCFT) introduced as early as 1906 by Mueller & Oppenheimer (9) and adapted to clinical practice by Kristiansen (7), Price (10) and Torrey (15) is still one of the few tests available for clinical purposes. There are, however, conflicting findings regarding the sensitivity and specificity of this test. Its clinical significance was recently evaluated by Magnusson & Kjellander (8), Watt et al. (17), Ratnatunga (11), Danielsson et al. (4) and by Rodas & Ronald (13). The diagnostic value of the test in complications to gonorrhoea was stressed by Magnusson & Kjellander (8) and by Danielsson et al. (4). The results obtained by these authors and also by the others are, however, more conflicting in patients with uncomplicated gonorrhoea. This lack of unanimity may be due to a variety of reasons, such as differences in the antigenic set-up of the gonococcal strains, whether the patients have been previously infected or not, and variations in the test procedures. In order to examine some of these variables we have performed an investigation on the GCFT with a standardized test procedure on sera from well controlled patient groups. We found it of particular interest to examine the extent to which the selection of gonococcal strains for antigens could influence the results.

MATERIAL AND METHODS

Patients and Serum Specimens

Serum specimens were obtained from three groups of patients as follows:

Patient group 1

During a 2-month period (November–December, 1972) serum specimens were collected from 157 females consecutively attending the venereological outpatient clinic at Södersjukhuset, Stockholm. An additional serum specimen was obtained 2 weeks after the first one from 79 patients.

All the patients were interviewed according to a fixed questionnaire. Information about previous gonorrhoea or present exposure to gonococcal infection as well as on symptoms of a suspected current infection was registered. Specimens were taken from the patients from the urethra, cervix and rectum for culture of gonococci according to standard procedures. A diagnosis of gonorrhoea was based on the growth of oxidase-positive, Gram-negative diplococci, fermenting glucose—but not maltose or levulose. None of the patients with gonorrhoea had any symptoms indicating complications to their gonococcal infection.

Patient group 2

Serum specimens were collected during a 3-month period (July–October, 1973) from 54 patients, 21 females and 33 males respectively, with an established bacteriological and clinical diagnosis of uncomplicated gonorrhoea. The patients attending the venereological outpatient clinic at Södersjukhuset, Stockholm, were investigated and interviewed as patient group 1 and the serum specimens were collected before antibiotic treatment was instituted.

Patient group 3

Serum specimens, obtained during a 3-year period (1971–1973) from 35 patients with disseminated gonococcal infection, were included in comparative GCFTs. These patients were from the Örebro area and the diagnosis of disseminated gonococcal infection was based on clinical and bacteriological findings as described elsewhere by Barr & Danielsson (2, 3). The specimens examined were taken during the convalescent phase of their disease.

Strains of N. gonorrhoeae

T1 or T2 colonies of *N. gonorrhoeae* strains (6) were isolated from patients with uncomplicated gonorrhoea, and selected for the preparation of three sets of polyvalent gonococcal antigens, A, B and C respectively, as follows.

Twelve gonococcal strains were isolated from patients attending the out-patient clinic at Södersjukhuset. These strains, six from males and six from females, were collected during a 3-month period (June–August, 1973) and selected on the basis of low anticomplementarity effect. The two sets, A and B respectively, were made up of strains from 3 males and 3 females each. The third set, C, was made up of gonococcal strains isolated from 6 patients, 3 males and 3 females, in the Örebro area.

These strains were collected during a 2-week period in July 1973.

Preparation of Antigens

Gonococcal whole cell antigens were used in the GCFT and prepared as follows. From each strain, T2 colonies (6) were seeded on GC agar plates and cultured for 22–24 hrs at 35–36°C in 5% CO₂ atmosphere. Each of the strains constituting antigens A and B was cultured on GC agar supplemented with horse serum and heated horse blood, and each of the strains constituting antigen C on BBL GC agar base supplemented with IsoVitalax and Bacto haemoglobin. The growth of the strains was harvested in sterile saline, washed once and then suspended in sterile Veronal buffer (VB), pH 7.2, to a concentration of 25 mg (wet weight) per ml. Each suspension was heated at 60°C for 30 min. The optimal antigen concentration for the complement fixation (CF) tests was estimated by chessboard titrations in microplates (see below) of various dilutions of the gonococcal suspensions against a human serum of known titre. Concentrations of 1.0–2.0 mg/ml (wet weight) were found to be optimal. The suspensions were pooled to constitute the antigens A, B and C described above. They were stored in aliquots at –30°C or –57°C until the day of use, and added at concentrations of 1.5 mg/ml in the test.

Estimation of Stability of Antigens

An antigen composed of two strains from antigen A and three from antigen B grown on a different medium was tested 2 years later against group 2 sera. With this antigen 19 of 39 sera were found positive at a dilution of 1/4 as compared with 15/39 with antigen B (coefficient of correlation $r=0.89$). Thus it is clear that the antigenic properties of given strains can be preserved even after prolonged subcultivation of T1 and T2 colony types.

Complement Fixation Test

The micro-modification of the Laboratory Branch Complement Fixation (LBCF) (16) test was used as described elsewhere by Danielsson et al. (4). Serum specimens were heat-inactivated at 56°C for 30 minutes and twofold diluted in VB buffer, pH 7.2, starting at an initial dilu-

Table 1. *Sera from patient group 1*

Consecutive female patients attending the Department of Venereology, grouped according to lowest GCFT titre obtained with the antigens A, B and C

GCFT titre	Culture positive	Culture negative			
		Prior gon.	No prior gon.	Total	Total
>1/2	29	20	61	81	110
1/2	8	3	2	5	13
≤1/4	24	6	4	10	34
Total	61	29	67	96	157

Table II. Analysis of female patients with a positive GCFT but negative culture for *N. Gonorrhoeae*

Patient number	First serum GCFT titre ^a to antigen			Second serum GCFT titre ^a to antigen			Prior gonococcal infection		Remarks
	A	B	C	A	B	C	Years ago	Number	
7	4	2	0	2	2	0	0	0	Partner gonorrhoea Obtained medicine for discharge
54	8	8	4				0	0	
79	4	8	0				1	>3	Salpingitis 6-7 times
91	2	4	0	4	4	0	2	1	
107	0	16	0				0	0	Taken metronidazole for discharge Taken medicine for discharge. Partner gon.
134	4	4	0	2	4	0	0	0	
141	4	4	2				1	1	Twice gon. salpingitis
146	4	4	0	4	2	0	2	>3	
150	16	16	0				2	>3	
157	8	8	0				3	1	
23	2	2	0				3	1	
34	2	2	0	2	2	0	6	1	
56	2	2	0	0	0	0	0	0	Direct microscopy pos. Treated at first visit
95	2	0	0				0	0	
144	2	2	2	0	2	2	3	1	

^a Reciprocal GCFT titre.

tion of 1:2. Antigen, guinea pig complement, and hemolytic system were added as recommended in the LBCF test procedure (16). The dilution of a serum specimen that gave a haemolysis inhibition of 70% or more was regarded as the final titre. A positive control serum with a titre of 1:64 to 1:128 was used throughout the investigation to check the quality of the antigens. Other definitive controls as proposed in the LBCF test procedure were always included.

RESULTS

Sensitivity and Specificity of the GCFT in Uncomplicated Gonorrhoea

Sensitivity

It will be seen from Table I that 61 of the 157 females in patient group I had a culture-positive gonorrhoea, clinically considered as uncomplicated. Twenty-four (39%) of these females had a positive GCFT at a dilution of $\leq 1/4$ with at least one of the antigens. However, 10/96 (10%) of the females without a current gonococcal infection had a GCFT of $\leq 1/4$. If a GCFT titre of $\leq 1/2$ was regarded as positive, the corresponding figures would be 32/61 (52%) and 15/96 (15%) respectively.

Specificity

An analysis of these 15 "false"-positives (GCFT titre $\leq 1/2$) is presented in Table II. It will be seen that 9 (60%) of these patients had had a previous

gonococcal infection, as compared with 20 out of the 81 (25%) patients without current infection and negative GCFT ($p < 0.02$). Another 5 patients had either clinical (direct smear positive but culture negative), epidemiological (partner gonorrhoea), or circumstantial (treated due to discharge prior to diagnosis) evidence of current gonococcal infection as compared with 5 in the group of 81 patients without current infection and negative GCFT.

It should also be noted (Table I) that among the patients without current gonococcal infection there

Table III. Results with different antigens in patient group I

GCFT positive sera. Antigens A and B are each composed of six strains from the Stockholm area, and antigen C of six strains from the Örebro area. Patient group I sera were obtained from female patients with uncomplicated gonococcal infection

GCFT result with antigen			Number of GCFT-positive sera	
A	B	C	titre $\leq 1/2$	titre $\leq 1/4$
Pos.	Pos.	Pos.	17	13
Pos.	Pos.	Neg.	22	13
Pos.	Neg.	Neg.	3	4
Neg.	Pos.	Neg.	5	4
Total			47	34

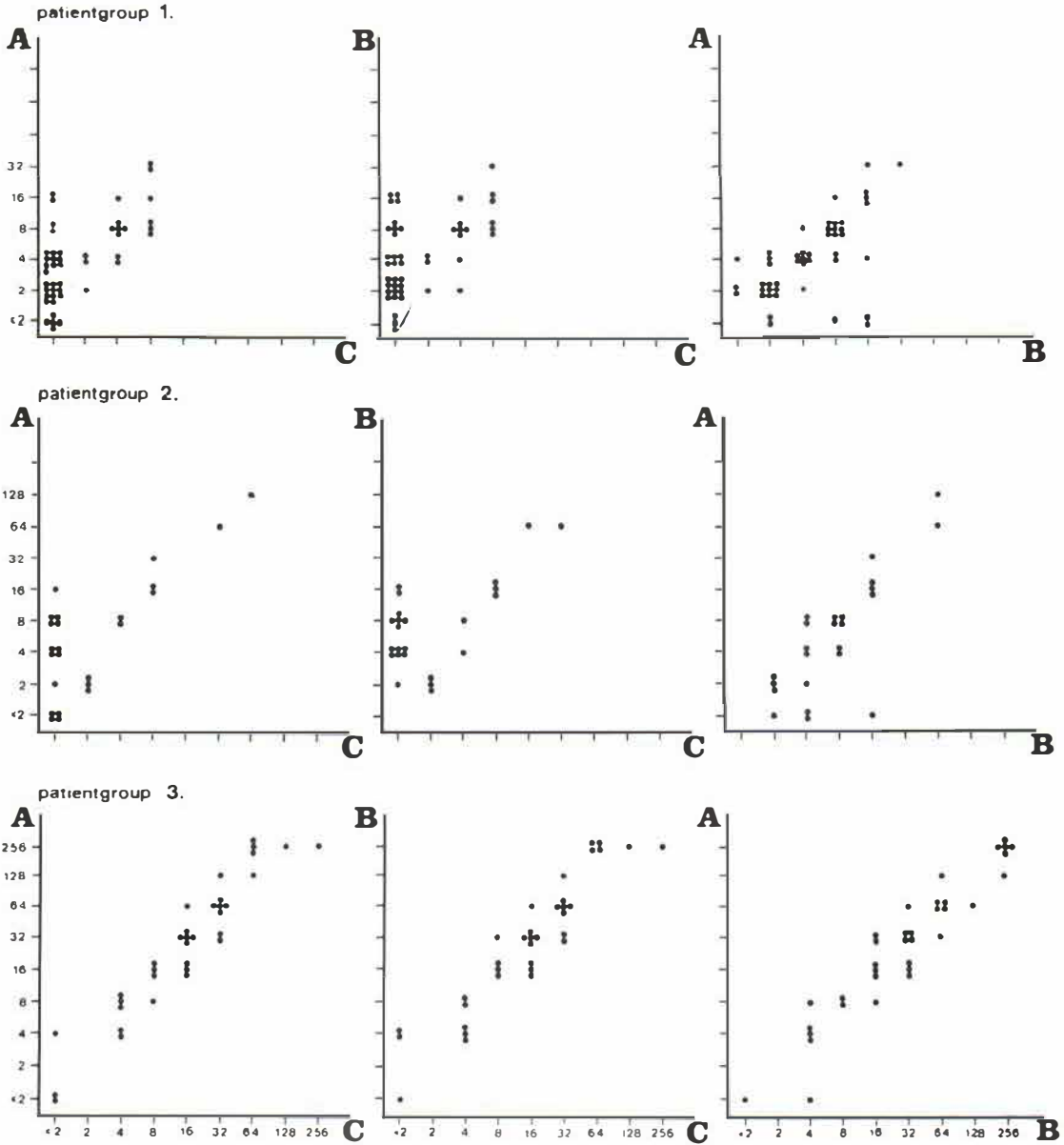


Fig. 1. Comparison between different antigens in the GCFT in patient groups 1, 2 and 3. Antigens A and B are composed of six strains from the Stockholm area, and antigen C of six strains from the Örebro area. Patient groups 1 and 2: sera from patients with uncompli-

cated gonococcal infection from the Stockholm area, with an interval of 9 months. Patient group 3: sera from patients with disseminated gonococcal infection from the Örebro area. On the x and y axis, reciprocal GCFT titres.

was a positive GCFT in 6/29 (21%) of those with a history of a previous infection as compared with 4/67 (6%) of those without. These figures were 9/29 (31%) and 6/67 (9%) respectively when positive GCFTs were also considered at a dilution of 1/2. This difference is significant ($p < 0.02$).

Convalescent sera

An analysis of the 79 "convalescent phase" sera yielded little further information. One patient with a present gonococcal infection showed a twofold rise in titre and another 4 turned positive (2 with serum titres of 1/4 with both antigens A and B, and 2 with

titres of 1/2 to a single antigen). Two patients without current gonococcal infection also turned positive with serum titres of 1/4 and 1/2 respectively with a single antigen (B).

Antigens

The diagnostic yields in patient group 1 with the three gonococcal antigen sets are compared in Table III. It will be seen that of the total number of positives with titres $\leq 1/2$ antigens A and B picked up 89.5% and 93.5% respectively, with agreeing results in 83%. Antigen C, however, only picked up 17/47, i.e. 36%, which is statistically significant ($p < 0.001$) as compared with antigens A and B.

Comparison of Diagnostic Yields with Three Gonococcal Antigens in Patients with Uncomplicated and Complicated Gonorrhoea

The diagnostic discrepancies observed in patient group 1 between antigens A, B and C were further investigated with regard to serum titres in patient groups 1 and 2 with uncomplicated gonorrhoea, and patient group 3 with disseminated gonococcal infections. The results from these comparative studies are presented in Fig. 1. In patients with complicated gonorrhoea, antigen C gave the same distribution pattern of serum titres as antigens A and B. In this patient group the correlation coefficient for antigens A and C will be $r=0.95$, for B and C $r=0.93$, and for A and B, $r=0.95$.

The distribution of serum titres in patient group 1 is much the same for all antigens with rather low correlation coefficients (A/C, $r=0.66$; B/C, $r=0.50$, and A/B, $r=0.60$). It can be seen, however, that a number of sera will react with only one of the antigens, suggesting that these patients had been exposed to a strain with antigens that might have been present in only one of the antigen sets used in the test. The common antigen would then be better represented by the sera reacting with the two antigens being compared. With this selection, a closer correlation is indeed observed (A/C, $r=0.73$; B/C, $r=0.68$, and A/B, $r=0.87$) and furthermore the slopes of the regression lines will then be close to 1.0 with the same scale on the axis resembling closely the slopes observed in group 3 sera group 1: A/C $b_{y/x}=1.06$, B/C $b_{y/x}=0.99$ and A/B $b_{y/x}=0.87$, the corresponding values before the selection being 0.74, 0.55 and 0.60 respectively.

No difference was observed between group 1 sera and group 2 sera obtained in Stockholm with an interval of 9 months.

DISCUSSION

Specificity and sensitivity of the GCFT have been shown to be high in patients with complications to gonorrhoea (2, 3, 4) while they have been questioned by many authors in cases of uncomplicated gonorrhoea (for references, see 5, 8, 12, 14). There are undoubtedly unspecific reactions both in venereological and other groups of patients (1). According to Magnusson & Kjellander (8) they seem to play a minor role. This opinion is in agreement with the findings of the present work on a group of venereological patients in whom a positive GCFT was associated with a past or current gonococcal infection. The persistence of weak reactions for more than one year in some patients is, however, a drawback from a diagnostic point of view. As shown earlier by Danielsson et al. (4), a second serum specimen (in the convalescent phase) is of great diagnostic value in patients with complicated gonorrhoea, whereas it adds no further information in cases of uncomplicated gonorrhoea.

Gonococcal serology is hampered by the fact that we still do not have an accepted serotyping schema for *Neisseria gonorrhoeae*. In other words, we do not know what gonococcal strains should be selected for serological tests. The results of the present investigation showed that the random selection of gonococcal strains used for the preparation of antigens will to a large extent influence the results obtained by a serological test such as the GCFT. This may well explain the many conflicting results reported in literature and illustrated in the present investigation regarding the sensitivity of this test. Thus, one of the pooled antigens gave less than half of the total number of positive GCFTs obtained with the other two pools of antigen, despite the fact that the same number of gonococcal strains were included in all three antigenic pools.

These discrepancies can be explained by the fact that antigens A & B were prepared from gonococcal strains from the same geographical area as the patients from whom the serum specimens were obtained, whereas antigen C was from another geographical area 200 km away. The findings indicate that immune responses probably occur against strain-specific antigens in many patients with un-

complicated gonorrhoea. In contrast to these findings, all three antigens gave the same results in patients with disseminated gonococcal infections. This could be due to an immune response against common gonococcal antigens in these groups of patients. However, there are even other explanations and the serological findings in the present work stress the urgent need for antigenic characterization of *Neisseria gonorrhoeae*. Such work is now in progress.

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