Antibodies to gonococcal pili in women with asymptomatic gonorrhoea

Diagnostic value of the ELISA for testing women attending an STD clinic

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SUMMARY The diagnostic efficacy of an enzyme-linked immunosorbent assay (ELISA) using gonococcal pili 6650 as antigen was studied in asymptomatic women attending an STD clinic. Of the 182 women studied, gonorrhoea was diagnosed in 29%. The value of the ELISA was calculated on the basis of four arbitrary cut-off points in the test. The best predictive values for a positive result (PV(+)) were obtained at an absorbance value of $A \geq 1.15$ and $A \geq 1.30$ and that for a negative result at $A \geq 0.85$. When patients with a history of gonorrhoea were excluded, the PV(+) rose only at $A \geq 1.15$ (not at $A \geq 1.30$) and the PV(-) rose slightly. To be of use in the diagnosis of gonorrhoea in asymptomatic patients the ELISA should be used as follows: the result is positive at $A \geq 1.15$ and negative at $A < 0.85$, the PV(+) then being 0.52 and the PV(-) 0.85. Whenever sera give a result between $A = 0.85$ and $A = 1.15$, the test should be repeated.

Introduction

Women with asymptomatic gonorrhoea constitute a source from which fresh infections can readily arise. Serological tests using gonococcal pili as antigen to detect gonorrhoea give reliable results in women with asymptomatic gonococcal infections.¹⁻⁴ Our previous studies showed that an enzyme-linked immunosorbent assay (ELISA) using gonococcal pili of strain 6650 as antigen attained a sensitivity of 75% in women with asymptomatic urogenital/rectal gonorrhoea.³ In this study we assessed the diagnostic efficacy of the ELISA using gonococcal pili 6650 as antigen if several absorbance cut-off points are arbitrarily used. Sera from women who had no symptoms referable to the genital tract and attended the sexually transmitted disease (STD) clinic of the Rotterdam University Hospital Dijkzigt were tested by the ELISA.

Patients and methods

Sera were collected from 182 asymptomatic women attending the STD clinic of the Rotterdam University Hospital Dijkzigt between 1 January and 15 December 1981. Female prostitutes were not studied. At each visit material was obtained from the cervix, urethra, rectum, and oropharynx and cultured for *Neisseria gonorrhoeae* on selective Thayer-Martin⁵ media. Suspect oxidase-positive colonies were identified as gonococci by Gram-staining and sugar fermentation tests. If all the culture results were negative, cultures were repeated after one week if possible. If they were again negative, a diagnosis of gonorrhoea was excluded. No patients had a positive Gram-stained smear and negative culture results. All patients were tested routinely for the presence of other STDs.⁶

GONOCOCCAL STRAIN AND PREPARATION OF PILAR ANTIGEN

Pili of strain 6650 (Rotterdam) were used to prepare the antigen. They were purified according to Hermodson *et al.*⁷

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TABLE I *Classification by diagnosis of 182 asymptomatic women attending an STD clinic*

Patient groups	No of patients		Total
	No previous history of gonorrhoea	Previous history of gonorrhoea	
1. With asymptomatic gonorrhoea	42	10	52
2. Contact of gonorrhoea with negative culture results	18	8	26
3. No contact with gonorrhoea and negative culture results	86	18	104
Total	146	36	182

TABLE II *Seropositive results at different ELISA absorbance cut-off points in 182 asymptomatic women*

Absorbance cut-off point	No (%) of positive sera from different patient groups*:		
	Group 1 (n = 52)	Group 2 (n = 26)	Group 3 (n = 104)
≥0.85	38 (73)	13 (50)	36 (35)
≥1.00	34 (65)	12 (46)	30 (29)
≥1.15	32 (62)	10 (38)	19 (18)
≥1.30	29 (56)	10 (38)	14 (13)

*Group 1—women with asymptomatic gonorrhoea; group 2—contacts of gonorrhoea with negative culture results; group 3—no contact with gonorrhoea and negative culture results

TABLE III *Sensitivity, specificity, and predictive values of a positive (PV(+)) and of a negative (PV(−)) result at different ELISA absorbance cut-off points in 182 asymptomatic women*

Absorbance cut-off point	Sensitivity %	Specificity %*	PV(+)	PV(−)
≥0.85	73	62	0.44	0.85
≥1.00	65	68	0.45	0.83
≥1.15	62	78	0.52	0.83
≥1.30	56	82	0.55	0.82

*Including contacts of gonorrhoea

ENZYME-LINKED IMMUNOSORBENT ASSAY

The ELISA procedure was carried out as described.^{3 8}

Results

PREVALENCE OF GONORRHOEA

Of the 182 women, 52 had asymptomatic urogenital/rectal gonorrhoea (table I); one also had oropharyngeal gonorrhoea. In addition, 26 women who were contacts of men with gonorrhoea had negative culture results. As a rule these patients were treated on the basis of their history. The remaining 104 patients neither had gonorrhoea nor were contacts of the disease. The prevalence of gonorrhoea was therefore 29% (52/182). Thirty-six (20%) women had a history of gonorrhoea (table I); previous gonococcal infections dated from a few months to several years. The prevalence of gonorrhoea in patients without a history of gonorrhoea was the same as that for the entire group, 29% (42/146).

ELISA ABSORBANCE VALUES

The ELISA absorbance values in the various groups of patients are shown in the figure. If an absorbance value of ≥0.85 (initially established as the cut-off point in this ELISA)³ is accepted as the boundary between positive and negative results, sera from 38 (73%) of 52 women with asymptomatic gonorrhoea were positive; the corresponding figures for contacts of gonorrhoea and for the remaining women were 13 (50%) of 26 and 36 (35%) of 104 respectively. At various other absorbance cut-off points the corresponding seropositive ELISA results were calculated (table II). Sensitivity* decreased and specificity† increased as the cut-off point was raised (tables II and III). A striking finding was that in the group of contacts of gonorrhoea the number of positive sera

$$\text{*Sensitivity} = \frac{\text{diseased persons with positive test}}{\text{all diseased persons tested}} \times 100\%$$

$$\text{†Specificity} = \frac{\text{non-diseased persons with negative test}}{\text{all non-diseased persons tested}} \times 100\%$$

TABLE IV Seropositive results at different ELISA absorbance cut-off points in 146 asymptomatic women without a previous history of gonorrhoea

Absorbance cut-off point	No (%) of positive sera from different patient groups*:		
	Group 1 (n = 42)	Group 2 (n = 18)	Group 3 (n = 86)
≥0.85	31 (74)	9 (50)	23 (27)
≥1.00	28 (67)	9 (50)	18 (21)
≥1.15	26 (62)	9 (50)	11 (13)
≥1.30	20 (48)	9 (50)	9 (10)

*Group 1—women with asymptomatic gonorrhoea; group 2—contacts of gonorrhoea with negative culture results; group 3—no contact with gonorrhoea and negative culture results

TABLE V Sensitivity, specificity, and predictive values of a positive (PV(+)) and of a negative (PV(-)) result at different ELISA absorbance cut-off points in 146 asymptomatic women without a previous history of gonorrhoea

Absorbance cut-off point	Sensitivity %	Specificity %*	PV(+)	PV(-)
≥0.85	74	69	0.49	0.87
≥1.00	67	74	0.51	0.85
≥1.15	62	81	0.57	0.84
≥1.30	48	83	0.53	0.80

*Including contacts of gonorrhoea

FIGURE ELISA absorbance (A) values in 182 asymptomatic women attending an STD clinic: group 1—patients with asymptomatic gonorrhoea; group 2—contacts of gonorrhoea with negative culture results; group 3—patients not reported as contacts of gonorrhoea and without the disease.

did not diminish evenly and, that even at the cut-off point of $A \geq 1.30$, 38% of these sera were still positive.

The predictive values of a positive result‡ was highest at $A \geq 1.30$ ($PV(+) = 0.55$), but it exceeded 50% even at $A \geq 1.15$ ($PV(+) = 0.52$). The predictive value of a negative result§ was highest at $A \geq 0.85$ ($PV(-) = 0.85$).

WOMEN WITHOUT A HISTORY OF GONORRHOEA
The results of the ELISA and their predictive values in asymptomatic women without a history of gonorrhoea are given in tables IV and V. When the results in tables II and IV are compared it is evident that the sensitivity was not influenced by previous gonococcal infections. The specificity increased slightly when women with a history of previous gonorrhoea were excluded (tables III and V). The predictive value of a positive result was highest at $A \geq 1.15$ ($PV(+) = 0.57$) and that of a negative result at $A \geq 0.85$ ($PV(-) = 0.87$) (table V).

Discussion

Serological tests using gonococcal pili as antigen are highly sensitive in women with asymptomatic gonorrhoea. Buchanan *et al*¹ attained a sensitivity of 89%

$$\ddagger PV(+) = \frac{\text{number of diseased persons with positive test}}{\text{total number of persons with positive test}}$$

$$\S PV(-) = \frac{\text{number of non-diseased persons with negative test}}{\text{total number of persons with negative test}}$$

with a radioimmunoassay and Oates *et al*² that of 85% with the same test. The incidence of asymptomatic infections in women with uncomplicated gonorrhoea attending an STD clinic is about 40-60%.⁹

The aim of this study was to determine the value of the ELISA 6650 in diagnosing gonorrhoea in asymptomatic women if four absorbance cut-off points were arbitrarily chosen in the test. With the aid of sensitivity, specificity, and prevalence figures the corresponding predictive values for a positive (PV(+)) and for a negative (PV(-)) result were calculated. The best PV(+) was attained at $A \geq 1.15$ and $A \geq 1.30$ and the best PV(-) at $A \geq 0.85$.

Of the patients studied, 20% had a previous history of gonorrhoea. When these were excluded, the PV(+) rose only at $A \geq 1.15$ (not at $A \geq 1.30$) and the PV(-) rose slightly. Thus, previously acquired gonorrhoea influences particularly the specificity of tests using pili and exerts a less pronounced influence on sensitivity, even if the previous infection was acquired several years before. This agrees with our earlier unpublished findings. There was a difference in specificity between patients who were contacts of gonorrhoea and the other patients with negative culture results. Young and Low¹⁰ also using an ELISA with gonococcal pili as antigen found more positive test results in women who were contacts of gonorrhoea than in the other women with negative culture results. Among contacts of gonorrhoea undoubtedly there were a few culture failures. These patients were often treated on the basis of the history, and in many cases, therefore, only one culture was performed before treatment.

In a retrospective study of 210 women with gonorrhoea (material only from the cervix and urethra was cultured), Evans¹¹ found that the diagnosis was missed at the first examination in 10%. Moreover, at least some gonococcal strains do not grow on the selective nutrient medium used here.⁶ Finally, false-negative results may be obtained as a result of irrigation of the vagina with bacteriolytic or bactericidal solutions and surreptitious use of antibiotics.

For use in asymptomatic women attending an STD clinic the ELISA 6650 can be interpreted as follows: the result is positive at $A \geq 1.15$ and negative at $A < 0.85$, at which absorbances $PV(+) = 0.52$ and $PV(-) = 0.85$. When results are between $A = 0.85$ and $A = 1.15$, a new blood sample should be taken and the test repeated. This modification of the ELISA provides some help in the diagnosis of gonorrhoea in asymptomatic women attending an STD clinic.

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