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Comparison of the Mikrogen multi-target ELISA with the Mikrogen recomLine immunoblot for the detection of *Chlamydia trachomatis* IgG antibodies in serum in infertile women

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ABSTRACT

Objectives: *Chlamydia trachomatis* (CT) IgG serology is used in many fertility clinics in order to estimate the risk for tubal factor infertility (TFI) in the fertility work-up. The predictive value for TFI of the currently used mono-target CT serology test should be improved. This study compares the performance of the new multi-target Mikrogen recomWell CT IgG ELISA with the Mikrogen recomLine CT immunoblot and visualizes distribution of individual antibodies in serum with the immunoblot in order to potentially improve the current CT IgG serology test that is clinically used.

Methods: Study population consisted of 183 Dutch Caucasian infertile women who underwent laparoscopy and/or hysterosalpingography. 48 women had TFI, 135 were controls. Serum was tested with Mikrogen CT IgG ELISA, which detects 3 CT IgG antibodies in one well, and Mikrogen CT immunoblot, which can individually detect 5 CT IgG antibodies. Tests were compared based on the results in general and in the case and control group also taking the individual antibodies into account. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), Kappa value and distribution of individual antibodies in positive samples were calculated.

Results: In 183 patients 51% tested positive in the ELISA versus 35% in the immunoblot. 32% versus 65% tested negative. Difference between PPV was not statistically significant (33% and 39% respectively) and NPV in both tests was 81%. Difference in sensitivity and specificity was statistically significant, respectively 65% vs. 52% and 54% vs. 71%. Kappa was only 45%. 64.5% of samples that tested positive with ELISA were positive for at least 4 individual CT antibodies with the immunoblot.

Conclusion: The concordance between CT ELISA and CT immunoblot is moderate. Due to separate criteria for positivity of both tests there is a significant difference in sensitivity and specificity. PPV and NPV, the most relevant characteristics for clinicians, of both tests did not differ significantly. The distribution of individual antibodies and the adjustment of the immunoblot algorithm will be further explored in the future in order to develop a potentially better prediction method for TFI with a higher clinical accuracy.

1. Introduction

Chlamydia trachomatis (CT) serology is used in epidemiological studies to determine the incidence and prevalence of CT in the population, and in clinical studies CT IgG is used to identify women at risk of CT complications such as pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility (TFI). In fertility clinics CT IgG assays are used in order to estimate the patient's risk for TFI (van Aar et al., 2014; Woodhall et al., 2017).

To assess the risk of TFI, the most commonly used commercial CT IgG test in Dutch fertility clinics is a mono-target ELISA (Medac IgG pELISA) that detects antibodies directed against the CT major outer membrane protein (MOMP). However, the predictive value for TFI of this MOMP ELISA is limited and there is a clinical unmet need for more accurate tests for the prediction of TFI (Land et al., 2003; Wills et al., 2009). In a study population of infertile women, the new Mikrogen multi-target ELISA has been shown to detect a higher percentage of TFI patients as CT IgG positive than the Medac MOMP ELISA. However, this

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difference was not statistically significant (van Ess et al., 2017). This Mikrogen CT serology test is an indirect sandwich ELISA that detects antibodies directed against MOMP as well as the highly purified translocated actin-recruiting phosphoprotein (TARP) and chlamydial protease-like activity factor (CPAF). TARP and CPAF are virulence factors expressed during CT infection and have immunodominant epitopes (Chen et al., 2012; Clifton et al., 2004; Yang et al., 2016). Within one Mikrogen multi-target ELISA well that tests positive, it is not possible to differentiate which antibodies are responsible for the positive test result. In addition to this multi-target ELISA, in order to differentiate between the antibodies in serum, Mikrogen developed an IgG immunoblot that detects antibodies directed against five CT epitopes: MOMP, TARP, CPAF, chlamydial heat shock protein 60 (cHSP60), and outer membrane protein 2 (OMP2). cHSP60 and OMP2 also play an important role in the pathogenesis of CT infection and are immunogenic proteins (Cappello et al., 2009; Hsia and Bavoil, 1996). Immunoblots visualize the antibody reactivity to each individual antigen, and potentially provide a broader picture of the antigenic response compared to currently used immunoassays. More knowledge about the predictive value for TFI of individual or combinations of antibodies is needed since more accurate tests will improve the accuracy of the fertility work-up.

This study compares for the first time the performance of the Mikrogen CT IgG recomWell ELISA with the Mikrogen recomLine Chlamydia immunoblot in the prediction of TFI in infertile women. Also the distribution of positive individual antibodies will be visualized with the Mikrogen immunoblot.

2. Methods

The study population consisted of 183 Dutch Caucasian infertile (i.e. not having conceived after at least one year of unprotected intercourse) women who attended the fertility clinic of the University Medical Center in Groningen (UMCG), the Netherlands, between 2007 and 2013. The 183 patients in this study were a selection out of 613 consecutive subfertile patients. As part of the fertility work-up 101 patients (55%) underwent laparoscopy and 82 patients (45%) underwent hysterosalpingography (HSG) to assess tubal patency. In this group of 183 patients 48 (26%) patients had laparoscopically verified TFI (defined as extensive adhesions and/or distal occlusion of at least one tube (Land et al., 1998)) and were considered as cases, and 135 patients did not meet the TFI criteria and were considered as controls. As part of the fertility work-up blood was drawn in all women and a chlamydia antibody test (CAT) was performed by Medac IgG pELISA. CAT positive patients were considered at high risk for TFI and underwent laparoscopy, while in CAT negative patients HSG was done. All spare sera were cryopreserved in −20 °C.

For this study spare serum samples were defrosted after two to eight years, and the Mikrogen recomWell ELISA and recomLine immunoblot (strip immune assay) were performed (Mikrogen GmbH, Neuried, Germany). ELISA tests were carried out and analyzed following the suppliers manual. Mikrogen ELISA samples were considered negative when < 20 U/ml and positive when > 24 U/ml. Samples between 20 and 24 U/ml were borderline. For the calculation of sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) ELISA borderline samples were considered negative.

For comparison and analysis of the individual antibodies the Mikrogen recomLine Chlamydia IgG immunoblot was used (Mikrogen GmbH, Neuried, Germany). This nitrocellulose strip immunoassay with recombinant species-specific antigens detects IgG antibodies against *C. trachomatis*, *C. psittaci*, and *C. pneumoniae*. In this study only antibodies directed against *C. trachomatis* antigens MOMP, TARP, CPAF, cHSP60 and OMP2 were taken into account. Work-up of the cryopreserved blood samples was according to the manufacturer's instructions. The immunoassay strips were analyzed with the "recomScan" software from Mikrogen, that classifies samples as positive, borderline, or negative based on an algorithm of summation of the individual antibodies that are present in a blood sample.

Table 1
Results of the Mikrogen recomWell CT IgG ELISA and the Mikrogen recomLine CT IgG immunoblot in 183 infertile patients.

Mikrogen ELISA	N	%	Mikrogen Immunoblot CT	N	%
CT IgG neg	58	32%	CT IgG neg	119	65%
CT IgG borderline	32	17%	CT IgG borderline	0	0%
CT IgG pos	93	51%	CT IgG pos	64	35%
Total	183	100%	Total	183	100%

Descriptive statistics were performed and presented as numbers and percentages and as a Venn-diagram. Kappa values were used to test for agreement between the two assays. Furthermore, we calculated the positive and negative predictive values (PPV and NPV), and sensitivity and specificity of both assays for TFI using MedCalc (MedCalc, 2018). *P*-values < .05 were considered statistically significant. Chi-square and McNemar tests were performed for the calculations of *p*-values.

3. Results

Of the 183 patients 51% tested positive in the ELISA versus 35% in the immunoblot, and 32% versus 65% tested negative (Table 1). In the ELISA 17% tested borderline, versus 0% with the immunoblot.

Within the 48 TFI patients the ELISA detected 31 (65%) as CT IgG positive and the immunoblot detected 25 (52%) as positive. In the control patients this was 46% and 29% respectively. In the patients with TFI 17 (35%) samples were negative with the ELISA and 23 (48%) samples were negative with the immunoblot (Table 2). The PPV of the ELISA and immunoblot was 33% and 39% respectively, with no statistically significant difference. NPV in both tests was 81%.

The ELISA had higher sensitivity (65%) than the immunoblot (52%), but the specificity was lower (54% vs. 71% respectively). The differences in sensitivity and specificity between both tests are statistically significant (for sensitivity $p = .04123$, for specificity $p = 1.083 * 10^{-5}$).

Of positive ELISA samples 32% were negative with the immunoblot. All negative ELISA results remained negative when analyzed with the immunoblot. Kappa was 0.45, which indicates a moderate concordance of both tests according to Landis and Koch (1977). Within the 48 cases with TFI the kappa value was 0.46 (moderate). Within the 135 infertile controls who did not meet the TFI criteria kappa was 0.42 (moderate). When the borderline ELISA samples were considered to be negative, the kappa value in the total study population ($n = 183$), TFI cases ($n = 48$) and controls ($n = 135$) was respectively 0.66, 0.75, and 0.62 (all substantial concordance). By considering borderline ELISA results as positive, the kappa value in the total population, TFI cases and controls was 0.40, 0.36, and 0.39 respectively (all fair concordance).

About two third of the samples that were positive with the ELISA were also positive for MOMP (65%), TARP (59%), CPAF (65%), and OMP2 (62%) antibodies (Table 3). For the cHSP60 antibodies this was 40%. In all but two samples that were positive for cHSP60 other antibodies were positive as well.

Fig. 1 shows the number of samples that are positive for one or more of the five antibodies that can be detected with the immunoblot. Of all combinations of positive antibodies in the immunoblot ($n = 76$) 36.8% of the samples were positive for all antibodies, and 18.4% were positive for all antibodies except cHSP60. Forty-five (59.2%) samples were positive for the combination of MOMP, TARP, and CPAF. These samples were also positive with the ELISA. Forty-nine (64.5%) samples were positive for at least four antibodies.

4. Discussion

The aim of this study was to compare the performances of the