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RESEARCH ARTICLE

Performance of the multitarget Mikrogen *Chlamydia trachomatis* IgG ELISA in the prediction of tubal factor infertility (TFI) in subfertile women: comparison with the Medac MOMP IgG ELISA plus

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One sentence summary: This study compares the predictive value for *Chlamydia trachomatis* induced tubal factor infertility of a mono-target CT IgG ELISA to a multitarget CT IgG ELISA in order to improve the fertility work-up.

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ABSTRACT

There is a need for more accurate *Chlamydia trachomatis* (CT) IgG antibody tests for tubal factor infertility (TFI) diagnostics. We evaluated the predictive value for TFI of Medac ELISA *plus* (MOMP) and multitarget Mikrogen ELISA (MOMP-CPAF-TARP). Based on Medac ELISA *plus* results, 183 subfertile women underwent either hysterosalpingography or laparoscopy to diagnose TFI. TFI was defined as extensive adhesions and/or distal occlusion of at least one tube. Women not fulfilling the definition of TFI served as controls. Serum was subsequently tested with Mikrogen ELISA and results were compared. 48 patients had TFI, 135 were controls. Mikrogen ELISA tested 125 patients positive/borderline of which 32% had TFI. Medac ELISA *plus* tested 77 patients positive/borderline of which 29.9% had TFI. Mikrogen tested 40 out of 48 TFI patients positive/borderline, Medac 23 out of 48. Kappa value was 0.34. PPV of Mikrogen ELISA and Medac ELISA *plus* were respectively 32% (95% CI 26%–39%) and 30% (95% CI 24%–37%), and NPV 86% (95% CI 81%–91%) and 76% (95% CI 70%–82%). Both tests were comparable in the prediction of TFI. However, Mikrogen ELISA had a higher NPV and might be more reliable in identifying patients without TFI. Kappa-value showed limited concordance between both tests.

Keywords: *Chlamydia trachomatis*; tubal factor infertility; tubal pathology; serology; ELISA; IgG

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INTRODUCTION

Over 80% of *Chlamydia trachomatis* (CT) infections in women run an asymptomatic course and thus will most likely not be treated (Lanjouw *et al.* 2016). Untreated CT infections can lead to severe complications (Rahm, Gnarpe and Odlind 1988; Morre *et al.* 2000), such as pelvic inflammatory disease (PID), ectopic pregnancy and tubal factor infertility (TFI) (Bjartling, Osser and Persson 2007; Price *et al.* 2016). It has been estimated that 6.7% of a 35-year-old women who at least once tested positive for CT will develop TFI (Low *et al.* 2006). Patients who are repeatedly exposed to CT, for example, in persistent infection or re-infection, have a higher risk of developing tissue damage in the upper genital tract (Patton, Sweeney and Kuo 1994; Darville and Hiltke 2010).

The pathogenesis of CT infection and its sequelae is multifactorial and determined by interactions between pathogen, host and (local) environmental factors (e.g. co-infections). Chlamydial antigens and bacterial load, the cytokine profile during infection, HLA subtypes and other host genetic factors are all determinants in the outcome of a CT infection (Morre, Karimi and Ouburg 2009; Asner *et al.* 2014; Menon *et al.* 2015). Cellular host responses triggered by CT infection contribute to both protective immunity and pathogenesis. Tissue damage of the upper genital tract emerges when the cell-mediated immune reaction persists for long or when a hypersensitivity response arises after infection (Patton, Sweeney and Kuo 1994; Finethy and Coers 2016). As a result of an (over)acting adaptive immune system, persisting species-specific IgG antibodies are formed. Antibodies against CT are more frequently found in women with tubal pathology as compared to women without tubal pathology (den Hartog *et al.* 2005; Tiitinen *et al.* 2006; Budrys *et al.* 2012; Ghosh *et al.* 2015). Therefore, CT IgG antibody testing (CAT) has been introduced in the fertility work-up to screen subfertile women for their risk of TFI (Broeze *et al.* 2011).

In the Netherlands, women with a positive CAT are considered at high risk for TFI and are offered a laparoscopy, which is considered the gold standard for diagnosing TFI (Coppus *et al.* 2007). In case of TFI, these women are referred for in vitro fertilization (IVF). In CAT-negative women, the risk of TFI is considered to be low and no invasive testing by laparoscopy is performed, but tubal status is assessed by hysterosalpingography (HSG). If HSG does not show any abnormalities, in most of these couples expectant management is proposed before IVF. The most frequently used test for CAT in the Netherlands is the species-specific ELISA *plus* from Medac (Medac GmbH, Wedel, Germany). The test accuracy of Medac ELISA *plus* for TFI however is not optimal. Up to 45% of women who are CAT positive do not have TFI at laparoscopy, while 10%–20% of women with a negative CAT do have tubal pathology (Land and den Hartog 2006). Therefore, the current fertility work-up leads to a considerable number of unnecessary invasive and expensive procedures in CAT-positive women without TFI or delayed IVF procedures in CAT-negative women with TFI. There is an unmet clinical need for a serological test for a previous CT infection that can predict TFI more accurately, and the newly developed multitarget Mikrogen ELISA (Mikrogen GmbH, Neuried, Germany) might have more favorable test characteristics as compared to Medac ELISA *plus*.

MOMP is the immunodominant antigen of CT and Medac ELISA *plus* uses MOMP peptides to detect CT IgG antibodies. Mikrogen ELISA also contains MOMP antigens and two additional antigens, i.e. translocated actin-recruiting phosphoprotein (TARP) and Chlamydial protease-like activity factor (CPAF). TARP and CPAF are virulence factors that are expressed during CT infection and are likely epitopes for antibodies. In previous studies, Medac pELISA was used for the prediction of TFI (Land *et al.* 2003), while in this study all samples were tested by ELISA Medac *plus*. Medac pELISA and Medac ELISA *plus* both employ the same MOMP-peptide, but Medac ELISA *plus* has an added calibrator and uses a single-point quantification for titer quantification, based on a lot-specific calibration curve. According to Medac's datasheets, concordance between the tests is 99% (MedacDiagnostics 2016).

In this study, the predictive value for TFI of Medac ELISA *plus* is compared to Mikrogen ELISA. We hypothesized that Mikrogen ELISA might improve the diagnostic accuracy of CAT in patients with CT induced TFI, because Mikrogen ELISA detects, besides the MOMP target, antibodies directed against virulence factors of the CT bacterium.

METHODS

Sample collection and definitions

The study was performed in Dutch Caucasian women who visited the fertility clinic of the University Medical Center Groningen (UMCG) between 2007 and 2013 because of subfertility (i.e. not having conceived after at least 1 year of unprotected intercourse). As part of the fertility work-up, blood was drawn in all women and CAT (Medac ELISA *plus*) was determined. All spare sera were cryopreserved in –20°C. After excluding couples with severe male factor subfertility, CAT-positive women were referred for laparoscopy with tubal testing. HSG was performed only in those CAT-positive women who had a relative contraindication for laparoscopy (e.g. body mass index > 35 kg/m^2). In CAT-negative women, HSG was performed, and when tubal occlusion or abdominal pockets were seen on HSG, patients were referred for laparoscopy to confirm the presence of TFI. In women with patent tubes on HSG, no additional testing was done because of the high negative predictive value of HSG (Ludwin *et al.* 2017). CAT-negative women who were considered at high risk for TFI due to endometriosis (based on history, physical examination and/or findings at ultrasound) were referred for laparoscopy immediately. Only women with available CAT result and who had undergone HSG and/or laparoscopy were included in this study. Patients who had undergone previous pelvic surgery (except for an uneventful appendectomy or Caesarean section) were excluded. No data had been systematically collected on previous sexually transmitted diseases and CT infections.

The 183 patients in this study were a selection of all CAT positives and TFI positives, and a selection of CAT negatives, TFI negatives and patients without abnormal HSG out of 613 consecutive subfertile patients. We chose a distribution of 1:2.5 for cases and controls. TFI was defined as extensive adhesions and/or distal occlusion of at least one tube (Land, Evers and Goossens 1998). Severe TFI (sTFI) was defined as bilateral extensive periadnexal adhesions and/or bilateral distal occlusions, and was thus a subgroup of the total TFI population of this study (Verweij *et al.* 2015). Controls were selected such that they had no abnormalities on HSG and/or did not fulfill the definition of TFI at laparoscopy.

The study population and its subgroups are given in Fig. 1. Between 2007 and 2013, 613 couples visited the fertility center in the UMCG, and in 183 women CAT results by Medac CT IgG ELISA *plus* (Medac GmbH, Wedel, Germany) and findings at HSG and/or laparoscopy were available. In 77 women CAT was positive, and in 106 CAT was negative, and these 183 women are referred to as group 1. Within group 1, a subgroup was selected of 101

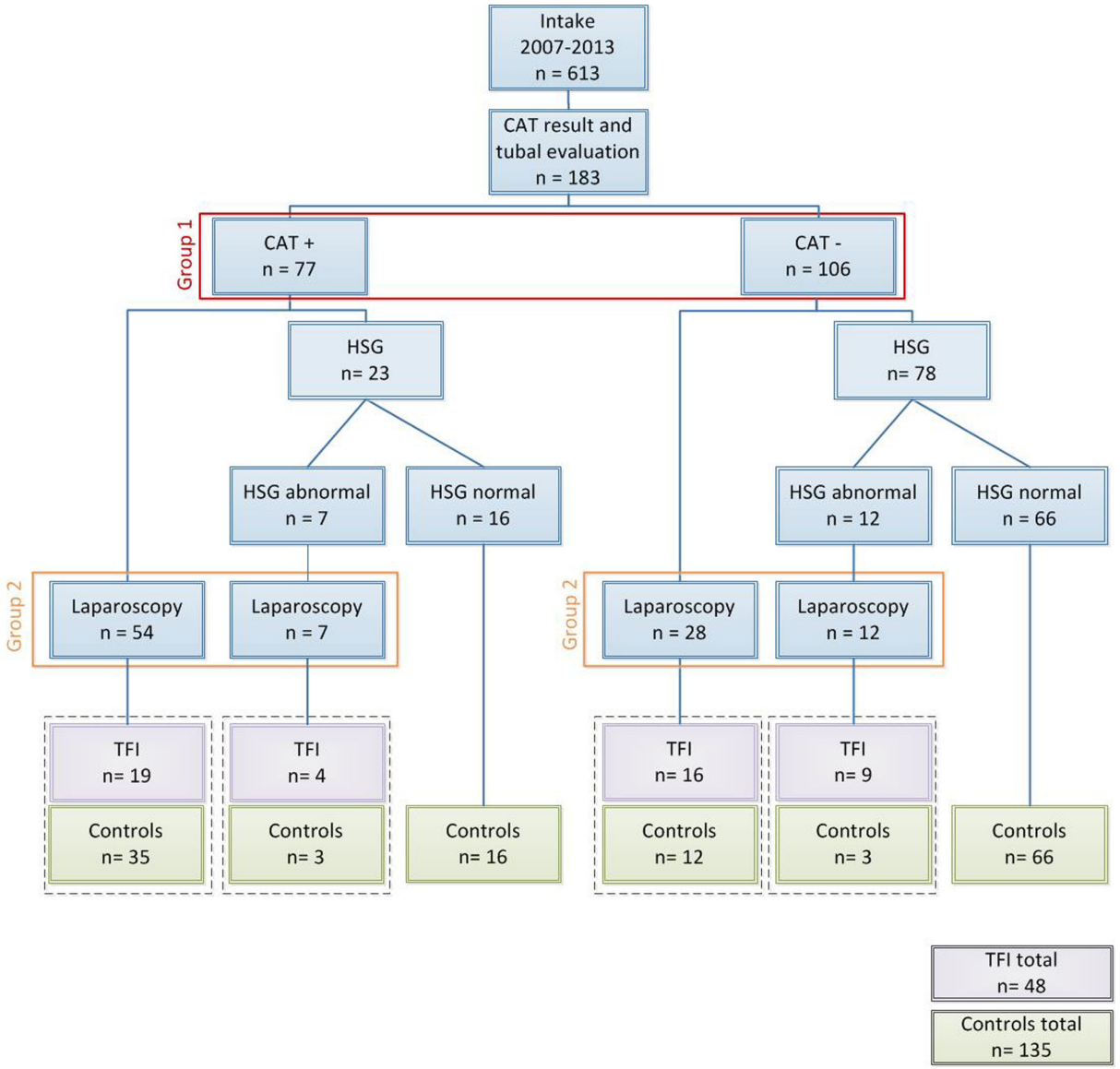

Figure 1. Flowchart of the study population. Group 1 (red box) consists of 183 patients who underwent either HSG or laparoscopy. Group 2 (orange boxes) consists of patients who underwent laparoscopy. HSG: hysterosalpingography. TFI: tubal factor infertility (extensive adhesions and/or distal occlusion of at least one tube). Controls were women who did not fulfill the criteria of TFI or had a normal HSG.

patients who had undergone laparoscopy and thus were proven TFI cases or controls. This subgroup of 101 patients is referred to as group 2 (Fig. 1). Another 101 patients underwent HSG, and 19 had an abnormal HSG and underwent laparoscopy, of whom 13 had TFI at laparoscopy. As Fig. 1 shows, in the total study population (group 1) 48 women were diagnosed as TFI (cases) and 135 women had no abnormalities on HSG or laparoscopy (controls). Within group 2, 48 patients had TFI and 53 patients had laparoscopically proven no TFI. In groups 1 and 2, the serological test performance of CT IgG Mikrogen ELISA and CT IgG Medac ELISA *plus* were compared.

Serological methods

During the fertility work-up, serum samples were tested for the presence of CT IgG antibodies with Medac ELISA *plus* (Medac GmbH, Wedel, Germany). Medac ELISA *plus* detects IgG antibodies directed against a CT-specific synthetic peptide of a variable domain from an immunodominant region of the MOMP. Medac ELISA *plus* tests were performed in the diagnostic laboratory of the UMCG according to the manufacturer's instructions. The cut-off for the Medac ELISA *plus* samples was 25 AU/ml and samples were considered borderline when the antibody levels were between 22 and 28 AU/ml. For clinical decision making and referral for HSG or laparoscopy, borderline results were considered negative.

For this study, spare serum samples were defrosted, and the *recom*Well ELISA from Mikrogen was used (Mikrogen GmbH, Neuried, Germany). This indirect sandwich ELISA detects antibodies to highly purified proteins MOMP, TARP and CPAF. The Mikrogen ELISA was performed on spare cryopreserved serum samples that had been stored for 2 to 8 years. 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. Borderline results with the Mikrogen ELISA and

Table 1. Results of Mikrogen ELISA and Medac ELISA *plus* in patients with TFI (n = 48) and controls (n = 135) in group 1 (all 183 patients).

	Patients				Patients		
Mikrogen ELISA	Total (n = 183)	Control (n = 135)	TFI (n = 48)	**Medac ELISA *plus***	Total (n = 183)	Control (n = 135)	TFI (n = 48)
Negative	58	50	8	Negative	106	81	25
Borderline	32	23	9	Borderline	14	11	3
Positive	93	62	31	Positive	63	43	20

Table 2. Concordance and discordance between results by Mikrogen ELISA and Medac ELISA *plus* in group 1 (n = 183).

		Medac				
		Negative	Borderline	Positive	Total	Kappa
Mikrogen	Negative	**52**	1	5	58	**0.34**
	Borderline	23	**3**	6	32	
	Positive	31	10	**52**	93	
	Total	106	14	63	183	

Medac ELISA *plus* were considered to be positive in the analyses in this study.

Ethical approval

Women attending the UMCG fertility clinic were offered a broad 'no objection' procedure and the participating women declared no objection for the use of their anonymized medical data and spare serum samples. This procedure was approved by the medical ethical board of the VU University medical center in Amsterdam.

Statistical analyses

Descriptive statistics were performed and results presented in tables and as a Venn diagram. The number of positive, borderline and negative test results in TFI cases and controls were compared between the Mikrogen ELISA and Medac ELISA *plus* for groups 1 and 2. Kappa values for groups 1 and 2 were calculated in order to quantify concordance between the two tests. A Venn diagram visualizes the overlap between the amount of positive results in both tests and TFI cases. The negative predictive value (NPV) and positive predictive value (PPV) of the two tests and their confidence intervals were calculated. P-values < 0.05 were considered as being statistically significant.

RESULTS

Patients characteristics

The median age in the total study population of 613 patients at intake in the fertility center was 32 years (range: 18–41). The prevalence of laparoscopically proven TFI in the total population of 613 patients was 7.8%. The TFI prevalence in the study population of 183 patients used in this study was 26.2%.

Comparison of Medac ELISA plus and Mikrogen ELISA

Within group 1 (all 183 patients who underwent either laparoscopy or HSG), Mikrogen ELISA tested 125 samples positive or borderline, of which 40 (32.0%) were from patients with laparoscopically proven TFI and 85 (68.0%) from control patients. Medac ELISA *plus* tested 77 patients positive or borderline, of which 23 (29.9%) had proven TFI and 54 (70.1%) were controls (Table 1). Mikrogen ELISA found 73.9% more TFI as compared to Medac ELISA *plus*, but also found 57.4% more IgG positives and borderlines in the control group. The difference between the number of IgG-positive patients with TFI in both tests was not significant. In the analyses, borderline test results were considered to be positive, but the percentages of patients with TFI did not change significantly when borderline results were considered to be negative.

Mikrogen ELISA tested 58 patients negative, of which 8 (13.8%) did have TFI and 50 (86.2%) were controls. Medac ELISA *plus* tested 106 serum samples negative, of which 25 (23.6%) belonged to patients with TFI and 81 (76.4%) to controls (Table 1). In the control group, Mikrogen ELISA detected more IgG positive and borderline patients than Medac ELISA *plus*.

Table 2 shows the (dis)concordance between both tests within group 1. The kappa value between Mikrogen ELISA and Medac ELISA *plus* was 0.34, which was a fair-to-moderate concordance (Landis and Koch 1977).

Within group 2 (i.e. 101 patients who underwent laparoscopy), Mikrogen ELISA identified 82 patients as CT IgG positive or borderline, of which 40 (48.8%) had TFI and 42 (51.2%) were controls. Medac ELISA *plus* tested 61 patients positive or borderline, of which 23 (37.7%) had TFI and 38 (62.3%) were controls (Table 3). The difference between the number of IgG-positive patients with TFI in both tests is not significant. As in group 1, the percentages of detected patients with TFI did not change when borderline test results were considered positive instead of negative.

In group 2, Mikrogen ELISA classified 73.9% more TFI cases as CT IgG positive or borderline than Medac ELISA *plus* did, and identified four controls more as IgG positive than Medac ELISA *plus* did. Mikrogen ELISA tested 19 samples as negative of which 8 (42.1%) belonged to cases and 11 (57.9%) to controls. Medac ELISA *plus* tested 40 samples negative, of which 25 (62.5%) were TFI cases and 15 (37.5%) were controls. As in group 1, Mikrogen ELISA identified more women with IgG positive or borderline results than Medac ELISA *plus* in group 2.

Table 4 shows the (dis)concordance between both tests in group 2. The kappa value between Mikrogen ELISA and Medac ELISA *plus* was 0.32, which is a moderate concordance. Between two completely concordant tests, a Kappa value between 0.8 and 1.0 is to be expected (Landis and Koch 1977).

Figure 2 illustrates that results obtained by Mikrogen ELISA do not fully overlap those by Medac ELISA *plus*. Seventy-one (23 plus 48) samples have either a positive or borderline results in both ELISAs, of which 23 belong to TFI cases. Mikrogen ELISA detected 17 more positive or borderline samples in TFI cases than Medac ELISA *plus* did. Also Mikrogen ELISA detected 37 positive or borderline patients who did not have TFI. Medac ELISA *plus* detected 6 patients without TFI as positive or borderline.

Table 3. Results of the Mikrogen ELISA and the Medac ELISA *plus* in patients with TFI (n = 48) and controls (n = 53) in group 2 (all 101 patients who underwent laparoscopy).

	Patients				Patients		
Mikrogen ELISA	Total (n = 101)	Control (n = 53)	TFI (n = 48)	**Medac ELISA *plus***	Total (n = 101)	Control (n = 53)	TFI (n = 48)
Negative	19	11	8	Negative	40	15	25
Borderline	17	8	9	Borderline	7	4	3
Positive	65	34	31	Positive	54	34	20

Table 4. Concordance and discordance between results by Mikrogen ELISA and Medac ELISA *plus* in group 2 (n = 101).

		Medac				
		Negative	Borderline	Positive	Total	Kappa
Mikrogen	Negative	**15**	0	4	19	**0.32**
	Borderline	10	**2**	5	17	
	Positive	15	5	**45**	65	
	Total	40	7	54	101	

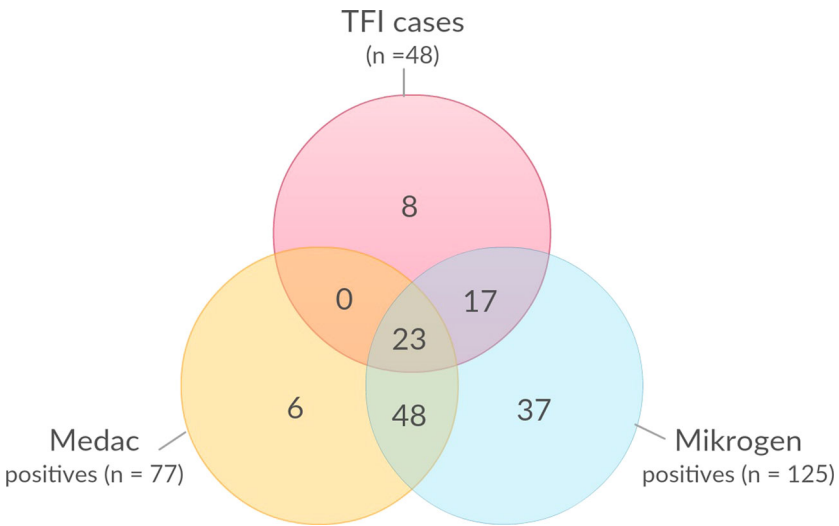

Figure 2. Venn diagram of overlapping numbers of TFI samples and positive Mikrogen ELISA and positive Medac ELISA *plus* results. Borderline test results were considered as positives.

Table 5. PPV and NPV of Mikrogen ELISA and Medac ELISA *plus* in 183 patients who underwent either HSG or laparoscopy (group 1) and the subgroup of 101 patients who underwent laparoscopy (group 2).

		PPV	95% CI	NPV	95% CI
Group 1	Mikrogen ELISA	32%	26%–39%	86%	81%–91%
	Medac ELISA *plus*	30%	24%–37%	76%	70%–82%
Group 2	Mikrogen ELISA	49%	39%–58%	58%	48%–67%
	Medac ELISA *plus*	38%	29%–47%	38%	29%–47%

Table 5 shows PPV for both tests in group 1 (all 183 patients) and group 2 (101 patients who underwent laparoscopy). For calculation of PPV and NPV, we considered the borderline results as positive. Differences in PPV and NPV for both tests in groups 1 and 2 were not statistically significant.

Ten TFI patients (20.8%) fulfilled the criteria of sTFI, i.e. bilateral extensive periadnexal adhesions and/or bilateral distal tubal occlusions. These 10 samples tested positive with Mikrogen ELISA with a titer ranging between 52.9 and 205.5 U/ml, which indicates that high titers are not predictive for sTFI per se. With Medac ELISA *plus*, 8 out of 10 sTFI patients tested positive. The two remaining patients tested negative and had CT IgG antibody titers of 4.1 and 6.3 AU/ml, respectively. The eight IgG-positive sTFI patients had titers between 30 and 342.5 AU/ml.

At the standard detection level of 20 U/ml, the Mikrogen ELISA detected 125 CT IgG-positive samples (40 TFI and 85 controls). Increasing the detection cut-off to 50 U/ml resulted in 58 CT IgG-positive samples (24 TFI and 34 controls), effectively increasing the PPV (from 32% to 41%) but actually detecting less TFI cases.

DISCUSSION

The aim of screening subfertile women by CAT is to identify those patients with previous CT infections who are prone to

develop late complications, e.g. TFI. Ideally, based on CAT, invasive tubal testing is selectively offered to patients at high risk for TFI only. Medac ELISA *plus*, based on MOMP-antibody detecting, is widely used for CAT, but its sensitivity and PPV are limited. We were the first to evaluate the predictive value for TFI of Mikrogen ELISA, a recently developed multi-antibody test targeting MOMP, TARP and CPAF antibodies. Compared to Medac ELISA *plus*, no significant improvement was found in the prediction of TFI by Mikrogen ELISA. Mikrogen ELISA detected almost all TFI cases, but detected patients with IgG antibodies who had no TFI as well. Medac ELISA *plus* on the other hand had found less IgG-positive patients without TFI, but identified only half of all TFI cases.

In our present study, the rates of CT IgG-positive TFI cases and CT IgG-positive controls with Medac ELISA *plus* were comparable to the results obtained in a previous study using the similar assay (Medac pELISA) in another population of subfertile women (Land *et al*. 2003). In this previous study, cases and controls were laparoscopically verified, which makes their study population similar to our group 2. den Hartog *et al*. (2005) however, found a higher amount of IgG-positive patients with TFI (54.2%) and lower amount of CT IgG-positive patients without TFI (7.9%), which may be due to using MIF as serological test for CAT, which is known to have a higher predictive value for TFI.

In our study, Mikrogen ELISA resulted in less CT IgG-negative TFI patients as compared to Medac ELISA *plus* (13.8% vs 23.6%). Therefore, the Mikrogen ELISA seems a more suitable assay for the identification of patients without TFI.

We observed that although PPV of both tests is low (32% and 30%), the NPV of the Mikrogen ELISA was 10% higher as compared to the NPV of the Medac ELISA *plus* (Table 5; not significant). In the literature, higher PPVs and NPVs, of respectively 95% and 70%, for TFI have been described. However, these values relate to MIF serology instead of ELISA serology (Keltz, Gera and Moustakis 2006; Lal *et al*. 2013). Land *et al*. (2003) described a PPV and NPV of the Medac pELISA for TFI of respectively 38% and 90% in subfertile women who underwent laparoscopy. In group 1 of our study, the PPVs and NPVs of the Medac ELISA *plus* were not comparable with those of Land *et al*. In group 2, which is similar to the population of Land *et al*., NPVs and PPVs of both tests were lower, except for the Mikrogen ELISA which had a PPV of 49% in group 2.

The fact that the Medac ELISA *plus* outcomes in this study differ from those in the study of Land *et al*. may be due to the relatively small size of our present population, or due to performing more laparoscopies in CAT-negative patients at high risk for not CT-related TFI. This difference seems not to be due to the different test that is used, since the concordance between the Medac pELISA and Medac ELISA *plus* is 99% (MedacDiagnostics 2016).

The NPV was higher for Mikrogen ELISA, but no significant differences were found as confidence intervals were overlapping. A larger sample size would be needed to confirm this finding. In clinical practice, a screening test with high NPV would be useful to rule out CT-related TFI in subfertile women.

Even though the PPV and NPV of both tests are comparable, it is remarkable that the kappa value did not exceed 0.34 (Tables 2, 4, 5). This indicates that the CT IgG-positive women identified by both tests were not the same women. We had expected that the positive samples of Medac ELISA *plus* would also be positive with Mikrogen ELISA since both tests detect antibodies directed against (parts of) MOMP. An explanation for this finding may be that Medac ELISA *plus* uses a synthetic peptide of an immune-dominant region of MOMP that differs from the synthetic MOMP immune-dominant region used in Mikrogen ELISA, and that both tests thus detect slightly different antibodies.

In this study, we observed 8 out of 48 TFI patients to test negative in both assays (Fig. 2). In these patients, TFI may have another, not-Chlamydia-related etiology, such as prior *Neisseria gonorrhoea* infection or endometriosis (Hart 2016). Therefore, even if highly accurate CT antibody testing is available, CAT cannot predict all cases of TFI. For optimal risk assessment in an individual patient, CAT result should be combined with patient history and findings at physical examination and ultrasound (Schachter *et al*. 1979; Broeze *et al*. 2012).

All 10 severe TFI cases in our study group were positive by Mikrogen ELISA, and Medac ELISA *plus* identified 80% of sTFI patients. Severe TFI was not necessarily related to high IgG titers. It needs to be confirmed in larger studies whether Mikrogen ELISA is more suitable than Medac ELISA *plus* in detecting high-risk patients for more extensive disease. Proper identification of severe TFI is very relevant in the clinical setting, as these patients have no chances to conceive spontaneously and should be referred to IVF without delay.

Our finding that not all subfertile women with positive CT IgG titers have TFI with laparoscopy may be explained by the fact that tubal pathology does not necessarily have to be visible as macroscopic damage of the tubes. Microscopic intraluminal damage of the mucosa may also impair the ability to conceive (Coppus *et al*. 2011; Keltz *et al*. 2013; Menon *et al*. 2016).

A limitation of this study is the potential introduction of bias. We compared the two tests with each other, with laparoscopic results as the gold standard. Laparoscopy-based selection has biased the TFI prevalence. Referral for laparoscopy was based on Medac ELISA *plus* test results, which might have caused verification bias. Due to this verification bias, most CAT-negative patients have undergone HSG instead of laparoscopy, and TFI might have remained unnoticed as HSG is a less accurate test in diagnosing adhesions and tubal occlusion. However, it is difficult to prevent selection and verification bias in a clinical study, since performing CAT and invasive, costly laparoscopies in all patients is not feasible.

In conclusion, the recently developed multitarget Mikrogen ELISA did not improve the PPV of CAT for TFI. However, Mikrogen ELISA may have additional value because of its higher NPV as compared to Medac ELISA *plus*, identifying subfertile women without TFI more accurately. In our study, the difference in NPV between the two ELISA tests was not statistically significant, and further research in a larger population should be done to confirm our findings. Further research should focus on testing of panels of chlamydial antigens by Mikrogen's recomLine IgG immunoblot including individual antigens such as TARP, CPAF, OMP2 and Chlamydia heat shock protein 60 (cHSP60) in order to potentially obtain a better risk assessment for TFI. Although an immunoblot is more time consuming, it may be valuable in the identification of TFI patients in a group of high-risk patients.

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