Diagnosing tubal pathology: The individual approach
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Chapter 4

Chlamydia antibody testing and diagnosing tubal pathology in subfertile women: an individual patient data meta-analysis

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Abstract

Introduction The Chlamydia IgG antibody test (CAT) shows considerable variations in reported estimates of test accuracy, partly because of the use of different assays and cut-off values. The aim of this study was to reassess the accuracy of CAT in diagnosing tubal pathology by individual patient data (IPD) meta-analysis for three different CAT assays.

Methods We approached authors of primary studies that used micro immunofluorescence tests (MIF), immunofluorescence tests (IF) or enzyme-linked immunosorbent assay tests (ELISA). Using the obtained individual patient data, we performed pooled ROC analysis and logistic regression analysis with a random effects model to compare the three assays. Tubal pathology was defined as any or as bilateral tubal obstruction.

Results We acquired data of 14 primary studies containing data of 6,191 women, of which data of 3,453 women were available for analysis. The areas under the curve (AUCs) for ELISA, IF and MIF were 0.64, 0.65 and 0.75, respectively (P value < 0.001) for any tubal pathology and 0.66, 0.66 and 0.77, respectively (P value = 0.01) for bilateral tubal pathology.

Conclusion In Chlamydia antibody testing, MIF is superior in the assessment of tubal pathology. In the initial screen for tubal pathology MIF should therefore be the test of first choice.
Introduction

Subfertility, defined as failure to conceive within 12 months despite regular unprotected intercourse, occurs in 10% of couples (Cahill and Wardle, 2002; Mosher and Pratt, 1991; Taylor, 2003). Besides ovulation disorders and sperm defects, tubal pathology is one of the main causes of subfertility. The prevalence of tubal pathology in subfertile couples ranges between 10% and 30% (Evers, 2002).

There are many tests to diagnose tubal pathology, of which Chlamydia IgG Antibody Test (CAT) testing, hysterosalpingography (HSG) and diagnostic laparoscopy (DLS) with chromopertubation are most often used (Broeze et al., 2009). Although guidelines on the fertility work-up are not concordant, CAT is often recommended as a first-line test (British national Collaborating Center for Women’s and Children’s Health, 2004; Dutch Society of Obstetrics and Gynaecology, 2004). Unlike HSG or diagnostic laparoscopy, CAT is non-invasive and inexpensive (Mol et al., 1997). When negative, CAT is thought to avoid unnecessary and invasive diagnostic testing, whereas in CAT positive women, further testing can be performed early, avoiding long term expectative management (den Hartog et al., 2008). A conventional meta-analysis reported that accuracy estimates of CAT for individual studies ranged between 21% and 90% for sensitivity and between 29% and 100% for specificity, with a summary ROC estimating the accuracy of CAT to be moderate and comparable to that of HSG (Mol et al., 1997).

Unfortunately, in the assessment of the diagnostic accuracy of CAT and the comparison of different assays over the full range of possible test results, conventional meta-analyses are impeded since they are based on data reported at study level and limited to reported 2x2 tables. Pooling data to estimate overall accuracy is complicated by the use of different assays and different, mostly unreported, cut-off values in the included studies. Original data underlying the publications possibly contain CAT results recorded in a continuous rather than a dichotomized way, as well as information on several CAT assays within a single study and could be used to compare the accuracies of different tests (Broeze et al., 2009).

The aim of this study was to assess the accuracy of several CAT assays in diagnosing tubal pathology, using continuous test results in an individual patient data (IPD) meta-analysis.

Methods

Literature search

In a previous meta-analysis on the accuracy of CAT, we identified all studies that compared CAT results to laparoscopy findings published until February 1996 (Mol et al., 1997). An updated search was performed in Medline and Embase, from 1996 to January 1st 2010, using the words ‘Chlamydia’, ‘Chlamydia trachomatis’, ‘Chlamydia antibody’, ‘CAT’ and ‘tubal pathology’, ‘tubal infertility’, ‘tubal disease’, ‘fallopian tube disease’, ‘tubal obstruction’ or
‘tubal occlusion’. Cross-references of the selected articles were hand-searched and checked for other potentially eligible articles. No language restrictions were made. Two independent reviewers (KAB and SFC) screened the electronic searches for eligible articles by reading the title and abstract. Subsequently, we asked the authors of the selected articles to examine the provisional study list to identify any additional studies they may be aware of. In this way, data from studies that were missed by our search criteria, or data that were not published at all, were eligible for inclusion. We also considered inclusion of studies with potentially relevant data that were excluded in the previous conventional meta-analysis due to the inability to extract a 2x2 table.

**Data collection**

For each of the eligible articles, we obtained contact information on the first, second or last author through Medline, Embase or the internet. We approached these authors by email to inform them about the IPD meta-analysis project and invited them to share their data in this collaborative project. If authors were willing to participate, they were provided with a more detailed study proposal, and asked to send their original database. We requested the complete database in the original data format, as to minimise the authors’ efforts going through their database to select the appropriate variables or to convert data into a specific format. Variables and categories needed to be adequately labelled within the dataset or in a separate data dictionary. Minimal requested data included the following variables: (anonymous) patient identifiers, CAT results and the results of laparoscopy (tubal pathology absent or present). CAT results of micro-immunofluorescence tests (MIF), immunofluorescence tests (IF) and Chlamydia trachomatis specific enzyme-linked immunosorbent assay tests (ELISA) were collected. These tests detect antibodies to Chlamydia trachomatis either by fluorescence of the elementary bodies of Chlamydia or by spectrophotometer detection of the complex of Chlamydia peptides and IgG antibodies (Land et al., 2003; Tuuminen et al., 2000; Wang and Grayston, 1970).

Tubal pathology was defined as either any tubal pathology or bilateral tubal pathology. Any tubal pathology was defined as at least unilateral occlusion of the fallopian tubes, with or without hydrosalpinges or peritubal adhesions. Bilateral tubal pathology was present when such abnormalities were seen in both tubes. The authors were asked to indicate whether tubal pathology was unilateral or bilateral, if possible. If authors had unpublished follow-up data available, they were asked to share these data as well. Ethical approval had been obtained by the original authors.

**Quality assessment**

The quality of the included studies was assessed according to the criteria of the QUADAS checklist (Whiting et al., 2003). Additional items were created for the description of selection criteria, execution of tests and the diagnostic strategy that was used. Completeness of the datasets was described, based on the availability of data on patient identifiers, diagnostic
test results and target disease. We compared the obtained data and the published results for consistency. Authors were contacted to confirm missing data or to check major discordant results. In addition, we organized a collaborators meeting, where authors clarified details of the original study designs and the tests performed. We used Review Manager (RevMan, Version 5.0. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2008) to summarize the quality indicators according to QUADAS for the included studies.

**Statistical analysis**
We merged the data into a summary database if variables were compatible. Incompatible data were recoded before adding to the summary database. Data on MIF, IF and ELISA were analyzed separately. In studies that reported on assays from different manufacturers, we analyzed the most often used assay.

First, we assessed the prevalence of both any as well as bilateral tubal pathology for the individual studies and for the complete set of included studies. We estimated heterogeneity in patient characteristics and prevalences between the included studies, using I-square (Higgins et al., 2003).

Second, we estimated sensitivity and specificity, based on the reported dichotomised CAT results per study.

Third, to correct for missing laparoscopies, thereby reducing verification bias, we performed multiple imputation per center. In the imputation step we assumed that, within one study, women that did not have a diagnostic laparoscopy had a tubal status comparable to the tubal status of women with similar CAT results, but who did have a diagnostic laparoscopy (Begg and Greenes, 1983; de Groot et al., 2008; van der Heijden et al., 2006). We performed five rounds of imputation, adjusted for center of origin and CAT result.

Subsequently, we performed a Receiver Operator Characteristics (ROC) analysis and calculated an area under the curve (AUC) for each individual study. The AUC was used to express the accuracy of a diagnostic test, representing the probability that a randomly chosen diseased patient is correctly ranked higher than a randomly chosen non-diseased patient. A perfect test, one with a perfect ROC curve, has an AUC of 1.0, whereas an uninformative test has an AUC of 0.5 (Hanley et al., 1982).

Data from all studies together were analyzed in two ways. Overall accuracy of the CAT assays was first estimated by ROC-analysis on the empirical pooled data. In this analysis raw data was pooled for women with and without tubal pathology across studies and the resulting distributions were used to generate AUC estimates, without adjusting for the heterogeneity across studies. Then, the overall accuracy was estimated and ROC curves were generated by ROC-analysis based on the predicted probabilities from a random effects logistic regression model. In the latter model we adjusted for the heterogeneity across studies, by including a random intercept that accounts for differences in prevalence of tubal pathology across studies, and by including a random effect, which accounts for differences in test performance across studies. We used ANOVA to test for significant differences in
accuracy between the three assays. A p-value below 0.05 indicated statistical significance in these analyses.
Data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Literature search and data collection
In the previous meta-analysis 23 studies had been included (Mol et al., 1997). Our current search for new studies on the subject published after February 1996 resulted in a total of 2,642 potential studies on Medline and 1,673 potential studies on Embase reporting on tubal pathology, of which 483 were overlapping studies. In total, 283 studies were identified as potentially eligible for inclusion. Of these studies, 164 did not contain diagnostic data on CAT results and/or laparoscopy. Therefore, 119 studies were eligible for inclusion. Checks of cross-references of selected articles identified no other studies. Of the 119 studies, 26 articles were reporting on previously published studies and for 24 studies the authors could not be traced, leaving 69 studies eligible for our IPD meta-analysis.
Of these 69 studies we contacted the authors and 38 responded. Twenty-four reported their data to be lost, whereas the other 14 authors provided their data (Anestad et al., 1987; Debattista and Gazzard, 2004; den Hartog et al., 2005; den Hartog et al., 2006; Guerra-Infante et al., 2003; Idahl et al., 2004; Land et al., 1998; Land et al., 2003; Land and den Hartog, 2006; Logan et al., 2003; Machado et al., 2007; Mouton, 2002; Ng et al., 2001; Steures et al., 2007; Svenstrup et al., 2008; Tiitinen et al., 2006; Toye et al., 1993; van der Steeg et al., 2008; Veenemans and van der Linden, 2002). Thirteen studies were single centre studies, whereas the largest study was a multi-centre study, in which data were collected from 38 centers (Steures et al., 2007; van der Steeg et al., 2008). In the analyses all data from this multicenter study were processed as originating from one study. A flow chart of the included studies is shown in figure I.
Data on 6,191 individual women from 14 studies were included in the summary database. For 2,872 women, out of 12 studies, continuous CAT results were available. For 581 women, out of three studies, data on two CAT assays per individual woman were available. For the remaining 2,738 women there were no continuous CAT results available. These women were excluded from the analyses. Two studies did not contain continuous CAT results and were also excluded from the analysis. Finally, data on 3,453 CAT results were available for the analyses. All included women were referred to a fertility clinic after minimal one year of unfulfilled child wish. Study characteristics of the included articles are listed in table I.
An overview of the methodological quality of the 14 included studies is shown in figure II. All 14 included studies clearly described their patient selection and the execution of CAT. In eight studies the diagnostic strategy followed was not described or unclear. In five studies not all women received the reference test (i.e. diagnostic laparoscopy), whereas in two studies it was unclear whether all women received laparoscopy and partial verification bias could be avoided.

For 13 studies the comparison of consistency between received data and the published results showed only minimal differences in mean female age and percentage of primary subfertility, which were ignored. One study reported on 295 women instead of 395 women that were included in the database. Therefore, the quality of the received data was considered satisfactory for all included studies.
Out of the 3,253 women 1,686 had CAT assessed with ELISA, 743 with IF and 824 with MIF. In 35% of women with a positive CAT result, and in 50% of women with a negative CAT result, laparoscopy had not been performed. In these 1,046 women missing data on laparoscopy were imputed.

The overall prevalences of any tubal pathology and bilateral tubal pathology as defined on diagnostic laparoscopy were 29% (95% CI 27%-30%) and 13% (95% CI 12%-15%), respectively. The mean female age, percentages of primary subfertility, median duration of subfertility and prevalences of tubal pathology in the individual studies are shown in table II. There was a high degree of variability across the studies in duration of subfertility, CAT

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Total women</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
<th>Study design</th>
<th>DLS performed</th>
<th>Diagnostic strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhattacharya</td>
<td>2003</td>
<td>207</td>
<td>women referred for tubal evaluation</td>
<td>- previous tubal testing - previous tubal surgery</td>
<td>cohort study (prospective)</td>
<td>yes</td>
<td>CAT → DLS</td>
</tr>
<tr>
<td>Van der Steeg/ Steures*</td>
<td>2007</td>
<td>3706</td>
<td>women referred for subfertility evaluation</td>
<td>- previous tubal testing - previous tubal surgery</td>
<td>cohort study (prospective) (multicenter)</td>
<td>yes (depending on center)</td>
<td>CAT - → No TT CAT + → HSG/ DLS</td>
</tr>
<tr>
<td>Ng</td>
<td>2001</td>
<td>110</td>
<td>women referred for tubal evaluation</td>
<td>- previous pelvic surgery - severe male factor</td>
<td>cohort study (prospective)</td>
<td>yes</td>
<td>CAT à DLS</td>
</tr>
<tr>
<td>Van der Linden</td>
<td>2002</td>
<td>395</td>
<td>women referred for subfertility evaluation</td>
<td>unknown</td>
<td>cohort study (prospective)</td>
<td>not standard</td>
<td>CAT - → HSG HSG + → DLS HSG - → EXP à DLS</td>
</tr>
<tr>
<td>Allan</td>
<td>2004</td>
<td>52</td>
<td>women referred for tubal evaluation</td>
<td>- ovulation disorders - severe male factor</td>
<td>cohort study (prospective)</td>
<td>yes</td>
<td>unknown</td>
</tr>
<tr>
<td>Mouton</td>
<td>2002</td>
<td>107</td>
<td>women referred for subfertility evaluation</td>
<td>unknown</td>
<td>cohort study (only subset of women were subfertility pts)</td>
<td>not standard</td>
<td>unknown</td>
</tr>
<tr>
<td>Land/ Den Hartog</td>
<td>2005</td>
<td>662</td>
<td>women referred for subfertility evaluation</td>
<td>- previous pelvic surgery</td>
<td>cohort study (prospective)</td>
<td>yes</td>
<td>CAT - → HSG CAT + → DLS HSG + → DLS HSG - → EXP à DLS</td>
</tr>
<tr>
<td>Idahl</td>
<td>2004</td>
<td>244</td>
<td>women referred for subfertility evaluation</td>
<td>unknown</td>
<td>cohort study (prospective)</td>
<td>not standard</td>
<td>CAT → HSS or DLS HSS + → DLS HSS - → no DLS</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Out of the 3,253 women 1,686 had CAT assessed with ELISA, 743 with IF and 824 with MIF. In 35% of women with a positive CAT result, and in 50% of women with a negative CAT result, laparoscopy had not been performed. In these 1,046 women missing data on laparoscopy were imputed.

The overall prevalences of any tubal pathology and bilateral tubal pathology as defined on diagnostic laparoscopy were 29% (95% CI 27%-30%) and 13% (95% CI 12%-15%), respectively. The mean female age, percentages of primary subfertility, median duration of subfertility and prevalences of tubal pathology in the individual studies are shown in table II. There was a high degree of variability across the studies in duration of subfertility, CAT
Figure II. Methodological quality of included studies

Table II. Overview of prevalences and patient characteristics of the included studies, with assessment of heterogeneity of data across studies (I² statistic)

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of women</th>
<th>Female age mean (5th – 95th percentile)</th>
<th>Primary subfertility %</th>
<th>Duration of subfertility median (range)</th>
<th>Prevalence tubal pathology (%)</th>
<th>CAT positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Any tubal pathology</td>
<td>Bilateral tubal pathology</td>
</tr>
<tr>
<td>Bhattacharya</td>
<td>207</td>
<td>30.7 (23 - 38)</td>
<td>56</td>
<td>2.0 (0.4 - 10)</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Van der Steeg/</td>
<td>3706</td>
<td>32.4 (25 - 39)</td>
<td>62</td>
<td>1.6 (0 - 12)</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>Steures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ng</td>
<td>110</td>
<td>31.9 (25 - 37)</td>
<td>75</td>
<td>3.0 (1 - 12)</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>Van der Linden</td>
<td>395</td>
<td>31.8 (19 – 44)</td>
<td>65</td>
<td>2.2 (0.2 - 13)</td>
<td>27</td>
<td>na</td>
</tr>
<tr>
<td>Allan</td>
<td>52</td>
<td>33.8 (27 - 42)</td>
<td>na</td>
<td>na</td>
<td>88</td>
<td>70</td>
</tr>
<tr>
<td>Mouton</td>
<td>107</td>
<td>34.7 (27 - 43)</td>
<td>69</td>
<td>na</td>
<td>8</td>
<td>na</td>
</tr>
<tr>
<td>Land/ Den Hartog</td>
<td>662</td>
<td>30.9 (24 - 37)</td>
<td>72</td>
<td>1.4 (0 - 9)</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Idahl</td>
<td>244</td>
<td>31.1 (24 - 39)</td>
<td>57</td>
<td>na</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>Tiitinen</td>
<td>166</td>
<td>32.9 (25 - 39)</td>
<td>na</td>
<td>na</td>
<td>37</td>
<td>na</td>
</tr>
<tr>
<td>Alves</td>
<td>55</td>
<td>30.7 (24 - 38)</td>
<td>na</td>
<td>na</td>
<td>58</td>
<td>44</td>
</tr>
<tr>
<td>Guerra-Infante</td>
<td>100</td>
<td>29.6 (21 - 39)</td>
<td>na</td>
<td>na</td>
<td>58</td>
<td>44</td>
</tr>
<tr>
<td>Toye</td>
<td>72</td>
<td>34.3 (26 - 42)</td>
<td>na</td>
<td>na</td>
<td>61</td>
<td>na</td>
</tr>
<tr>
<td>Ánestad</td>
<td>103</td>
<td>30.5 (24 - 38)</td>
<td>na</td>
<td>na</td>
<td>42</td>
<td>na</td>
</tr>
<tr>
<td>Svenstrup</td>
<td>212</td>
<td>29.9 (24 - 37)</td>
<td>na</td>
<td>na</td>
<td>16</td>
<td>na</td>
</tr>
<tr>
<td>Total</td>
<td>6191</td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Heterogeneity I²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5%</td>
<td>25%</td>
</tr>
</tbody>
</table>
positivity and prevalence of tubal pathology, which was due to heterogeneity rather than chance as indicated by high $I^2$ values between 67% and 84%.

Sensitivity and specificity of CAT for the diagnosis of tubal pathology, based on the reported, dichotomised, CAT results per study are shown in table III. For any tubal pathology sensitivity ranged between 12% and 91% and specificity ranged between 35% and 100%. For bilateral tubal pathology sensitivity was between 31% and 70% and specificity between 52% and 86%.

Both the individual, as well as the pooled AUCs for the diagnosis of any and bilateral tubal pathology are shown in table IV. The AUCs for the diagnosis of any tubal pathology in the individual studies ranged from 0.52 to 0.79 for the ELISA, from 0.54 to 0.78 for IF and from 0.63 to 0.80 for MIF. The AUCs for the diagnosis of bilateral tubal pathology ranged from 0.53 to 0.79 for the ELISA, from 0.60 to 0.63 for IF and from 0.77 to 0.78 for MIF. The empirical pooled ROC analysis showed AUCs for any tubal pathology of 0.57, 0.63 and 0.64 for ELISA, IF and MIF respectively. For bilateral tubal pathology the AUCs were 0.61, 0.63 and 0.75, for ELISA, IF and MIF respectively. The random effects logistic regression
Figure III. ROC curves for the accuracy of CAT (based on IPD random effects models).

Figure IIIA. Accuracy of diagnosing any tubal pathology

The lines replicate the accuracy of CAT based on the IPD meta-analysis, the summary ROC points are based on the conventional meta-analytic approach. Used cut-off values are 1.1 for ELISA, 1:32 for IF and 1:16 and 1:32 for MIF.

Figure IIIB. Accuracy of diagnosing bilateral tubal pathology

The lines replicate the accuracy of CAT based on the IPD meta-analysis.
analysis, in which we accounted for heterogeneity across studies, showed an AUC of 0.64 for ELISA, an AUC of 0.65 for IF and an AUC of 0.75 for MIF. The AUCs of ELISA and MIF, as well as the AUCs of IF and MIF differed significantly (see table IV). Repeating this analysis for bilateral tubal pathology showed AUCs of 0.66 for ELISA, 0.66 for IF and 0.77 for MIF, with significantly different AUCs between ELISA and MIF and between IF and MIF.

The ROC curves of the random effect logistic regression models for the three assays for the diagnosis of any tubal pathology and bilateral tubal pathology are shown in figure IIIA en
figure IIIB, respectively. The accuracy of MIF was clearly better than the accuracy of ELISA and IF over the full range of sensitivities and specificities.

Discussion

At present, there are several diagnostic strategies for women with suspected tubal pathology. These strategies differ among countries and among fertility centers in the same country and region. Despite clinical research done so far there is still inconsistency between fertility guidelines about the best diagnostic strategy and there is no consensus on which test should be initially used, or on the most effective or cost-effective sequence of tests (British national Collaborating Center for Women’s and Children’s Health, 2004; Dutch Society of Obstetrics and Gynaecology, 2004). In many fertility centers, CAT is used ahead of more invasive tests such as HSG or laparoscopy, whereas in other centers CAT is not used at all and all women are directly referred for HSG or even laparoscopy.

In this study we reconsidered the accuracy of three different CAT assays in diagnosing tubal pathology based on an individual patient data meta-analysis, containing data of 6,191 women referred for subfertility in fertility centers all over the world.

The results of this IPD meta-analysis showed that MIF has a moderate ability to discriminate between women with and without tubal pathology. For example, at a sensitivity of 74% MIF had a specificity of 66%, which represents 26% missed cases of tubal pathology and 34% of women incorrectly referred for invasive testing, respectively. Clinically, the optimal cut-off value of CAT depends on the individual context. In some women it will be more important to detect tubal pathology with high certainty, for example in older women who start IUI treatment. In other women it is more important to rule out tubal pathology in order to avoid unnecessary invasive testing, for example in couples with a good prognosis for spontaneous pregnancy who delay treatment for a while. The accuracy of MIF is significantly better than the accuracy of ELISA and IF. So far, only a few studies reported on the comparison of several assays and only one compared MIF to ELISA, showing a better performance of the MIF, with a diagnostic odds ratio twice as high as the ELISA test (Land et al., 2003). The analyses in this IPD meta-analysis were based on a large amount of continuous CAT data, not restricted by chosen cut-off values, increasing the consistency and statistical power of this result.

There was a slight difference in accuracy of all three tests for the diagnosis of bilateral tubal pathology and any tubal pathology. The definition of tubal pathology varies in literature. Some studies exclude adhesions or hydrosalpinges, others emphasize the difference between proximal and distal tubal pathology. Our definitions of tubal pathology included occlusion of the fallopian tubes, peritubal adhesions and hydrosalpinges. We did not discriminate between proximal and distal tubal occlusion, since clinical management and pregnancy chances are similar in both types of occlusion (Farhi et al., 2007).
In IPD meta-analyses the substantial number of missing values in the database, both at the study level, as well as on the individual patient level may provide some limitations. As shown in the flowchart, not all eligible studies could be included in the present meta-analysis. This was due to a lack of information about the authors, a lack of response from the authors or loss of primary data. Combining IPD with aggregate data could overcome the problem of missing studies. Since our primary interest was the use of continuous test results to assess accuracy estimates of CAT, we were not able to use aggregate data, in which continuous test results are not available. To be sure that the selection of women in the included studies was representative, we roughly compared the patient selections in the included studies with the patient selections in the studies that were not available for inclusion. We found no major differences between these patient selections. Also, inclusion of only a subset of eligible studies might lead to biased results, but only if studies that do not participate in the IPD collaboration show test accuracies other than those studies that do participate. Since test accuracy of the included studies varied substantially, we do not expect to find a completely different spectrum in the studies that were not included.

We included some case control studies in the present meta-analysis. In these studies, the cases were women referred for fertility work-up and therefore comparable to women included from cohort studies. The controls were excluded from the meta-analysis. Also, compared to the total amount of included women, the included ‘cases’ counted for only 6 percent. We are of opinion that they will not have substantial influence on the estimated accuracy (Rutjes et al., 2005).

Besides missing studies, also missing data at the patient level might be a problem in IPD meta-analysis. Of the 6,191 women in the database, we were able to use data of 3,453 women in the analyses. A comparison of the characteristics of these women to those that were excluded from the analyses showed no differences.

Missing laparoscopy results is another cause of missing data at the patient level. For 35% of women with a positive CAT result, laparoscopy had not been performed, whereas for 50% of women with a negative CAT results data on laparoscopy were missing. This partial verification happens easily in clinical practice since women with a positive CAT result more often received a laparoscopy compared to women with a negative CAT result. When these subsets of women are not comparable to each other and women with missing laparoscopy results were omitted from the analyses, verification bias would occur (de Groot et al., 2008; van der Heijden et al., 2006). To reduce this partial verification bias we decided to impute the missing laparoscopy results. We decided not to show results from an analysis restricted to women that have both had CAT and laparoscopy, since we would then assume that data were missing completely at random, while we know from clinical practice that CAT results might influence the decision to perform laparoscopy. Also, results from a complete case approach are expected to be more prone to verification bias and could therefore be misleading (van der Heijden et al., 2006; Moons et al., 2006).
At present, there is no consensus on the type of analyses that should be performed in a diagnostic IPD meta-analysis. In a previous study we described the execution of analyses that we performed in this IPD meta-analysis (Broeze et al., 2010). To be able to express the accuracy of the CAT, we used the area under the ROC curve (AUC). Although sensitivity and specificity might be more applicable measurements for clinicians, these could not be reported without using cut-off levels for the CAT results. To compare the results of the three different assays (MIF, IF and ELISA), without losing any information, we used the continuous test results and therefore reported the AUC. Although there are several CAT assays available worldwide, we only included these three assays, since they were most often used and reported in the original studies and enough data was available for inclusion in this meta-analysis. We used random effects logistic regression models, since pooling data for all women across studies without adjustments would ignore differences across study populations, which could have led to biased results. This was also shown in table IV in which the empirical pooled AUCs for MIF are inconsistent with the data and with the random effects model.

We found relatively low sensitivities of CAT, combined with high specificities. The latter is clinically very important, because this implies that CAT might avoid unnecessary, invasive testing in women without tubal pathology. On the other hand, the estimated sensitivities might be too low to correctly rule out tubal pathology, which implies that women with tubal pathology might remain undiagnosed (Leeflang et al., 2008).

The question whether CAT has clinical relevance as a test in the fertility work-up will also depend on the comparison of its accuracy with that of other diagnostic information, such as medical history. In a previous retrospective study it was shown that the combination of CAT and medical history taking had a better yield than either of these alone (Coppus et al., 2007). The added value of CAT over the information from medical history, physical examination and other diagnostic tests can be adequately analyzed with individual patient data and will be assessed in a subsequent study, based on this IPD project. This analysis of the added value of CAT to the pre-test probability of tubal pathology can be restricted to MIF, since this study shows MIF to be the CAT assay with the best discriminative possibility.

In conclusion, our study showed that MIF has a significantly better accuracy than IF and ELISA for the diagnosis of any tubal pathology. The accuracy of MIF showed a moderate ability to discriminate between women with and without tubal pathology. ELISA and IF showed only poor discriminative ability, therefore MIF should be the test of first choice in the initial screen for tubal pathology.
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