Screening strategies for tubal factor subfertility

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BACKGROUND: Different screening strategies exist to estimate the risk of tubal factor subfertility, preceding laparoscopy. Three screening strategies, comprising Chlamydia trachomatis IgG antibody testing (CAT), high-sensitivity C-reactive protein (hs-CRP) testing and hysterosalpingography (HSG), were explored using laparoscopy as reference standard and the occurrence of a spontaneous pregnancy as a surrogate marker for the absence of tubal pathology. METHODS: In this observational study, 642 subfertile women, who underwent tubal testing, participated. Data on serological testing, HSG, laparoscopy and interval conception were collected. Multiple imputations were used to compensate for missing data. RESULTS: Strategy A (HSG) has limited value in estimating the risk of tubal pathology. Strategy B (CAT→HSG) shows that CAT significantly discerns patients with a high versus low risk of tubal pathology, whereas HSG following CAT has no additional value. Strategy C (CAT→hs-CRP→HSG) demonstrates that hs-CRP may be valuable in CAT-positive patients only and HSG has no additional value. CONCLUSIONS: CAT is proposed as first screening test for tubal factor subfertility. In CAT-negative women, HSG may be performed because of its high specificity and fertility-enhancing effect. In CAT-positive women, hs-CRP seems promising, whereas HSG has no additional value. The position and timing of laparoscopy deserves critical reappraisal.

Keywords: Chlamydia trachomatis; hysterosalpingography; screening; serological test; tubal factor subfertility

Introduction

The reference standard for diagnosing tubal factor subfertility is laparoscopy with tubal dye testing, by which tubal patency and the presence of peri-adnexal adhesions and endometriosis can be assessed. It also provides additional information on uterine malformations. Laparoscopy has several disadvantages, e.g. it is an invasive and expensive procedure requiring general anaesthesia, and it holds a 1.5% risk of surgical complications (Chapron et al., 1998). Furthermore, operating facilities may not be easily available in every clinic. Owing to these disadvantages, laparoscopy is unsuitable for routine application in subfertile women on a large scale. Obtaining a reliable estimate of the risk of tubal pathology preceding laparoscopy would allow for selecting only high-risk patients for this procedure. The chance of finding tubal pathology at laparoscopy can be estimated preoperatively by applying screening tests. Among different countries, the preferred screening method to determine the risk of tubal pathology varies (Portuondo et al., 1984; Helmerhorst et al., 1995; Balasch, 2000; Mol et al., 2001). Commonly used and well-evaluated modalities to screen for tubal pathology in the fertility evaluation are serum Chlamydia (C.) trachomatis IgG antibody testing (CAT) and hysterosalpingography (HSG) (Swart et al., 1995; Mol et al., 1997, 2001).

CAT is a serological marker of a previous C. trachomatis infection, but does not reflect the course of the infection. Because mainly persistent C. trachomatis infections, rather than cleared infections, are associated with an increased risk of tubal pathology (Grayston et al., 1985; Patton et al., 1994), the combination of CAT and high-sensitivity C-reactive protein (hs-CRP), reflecting a previous C. trachomatis infection and persistence of the micro-organism, respectively, has been proposed as a valuable set of markers for identifying subfertile women at highest risk of persistent C. trachomatis infection and the ensuing tubal pathology (Den Hartog et al., 2005). In the present study, HSG is considered as a screening test (as opposed to a diagnostic test) because of its lower performance in diagnosing tubal pathology and predicting future fertility when compared with laparoscopy (Swart et al., 1995; Mol et al., 1999) and because at present, in
many clinics, HSG is used to differentiate between patients who qualify for further tubal testing and those who do not. The role of HSG in the investigation of the subfertile couple covers more than testing tubal patency only, because it also provides additional information on uterine malformations and intracavitary pathology.

No consensus exists on which screening test (or series of screening tests) is to be preferred for assessing the risk of tubal pathology in subfertile women. In the present observational study, we evaluated different screening strategies in predicting tubal pathology, in order to develop a useful screening strategy in the diagnostic work-up of subfertile couples. For this purpose, three different screening strategies comprising the test modalities CAT, hs-CRP and HSG were explored in a population of subfertile women, by constructing decision tables (Glasziou and Hilden, 1986) and by using laparoscopy as reference standard and the occurrence of a spontaneous pregnancy as a surrogate marker for the absence of tubal pathology.

Materials and Methods

Study population

The study population consisted of 642 women who visited the Maastricht University Medical Centre Fertility Clinic because of subfertility between December 1990 and May 2004, and who consecutively underwent tubal testing (HSG and/or laparoscopy) as part of their fertility evaluation. In all women, blood was drawn at their initial visit to measure *C. trachomatis* IgG antibodies using CAT. Spare serum samples were cryopreserved and stored. In patients with a negative CAT and an otherwise normal basic fertility evaluation, tubal status was evaluated initially by HSG. If HSG showed abnormalities, or if patients did not conceive in the 6 months following HSG, the fertility evaluation was concluded by laparoscopy with tubal testing using Methylene Blue dye. In patients with a positive CAT, no HSG was performed, but tubal status was evaluated primarily by laparoscopy. Patients with a history of previous pelvic surgery (except for an uneventful appendectomy or Caesarean section) or pelvic inflammatory disease, and patients with suspected severe endometriosis, based on history and findings at pelvic examination, directly underwent laparoscopy and were excluded from the present study.

In the Netherlands, for retrospective analysis of anonymized patient data and stored sera, no ethical committee approval is required. In the Fertility Clinic of the Maastricht University Medical Centre, all couples are informed at intake about possible use of their anonymized data and stored sera for research purposes, and a ‘no objection procedure’ is followed. Only patients who had not objected participated in the present study.

Chlamydia trachomatis IgG antibody testing

For the present study, stored serum samples of 475 patients were available and retested for CAT by using an enzyme-linked immunosorbent assay (ELISA) (*C. trachomatis* IgG PELISA, Medac, Germany). The test was performed according to the manufacturer’s instructions. The cut-off level used for a positive test was >1.1.

High-sensitivity C-reactive protein

In each of the 475 available stored serum samples, hs-CRP was determined using CRP ELISA (DiaMed Eurogen, Belgium). A high-sensitivity test was used in order to reliably detect low CRP concentrations. The test was used according to the manufacturer’s instructions. Hs-CRP levels between 1.0 and 10.0 mg/l were considered positive, whereas hs-CRP levels <1.0 and >10.0 mg/l were considered negative (Den Hartog et al., 2005).

Hysterosalpingography

In 424 patients, HSG was performed using oil-soluble contrast medium. The procedure was recorded on videocap, and radiographs were made during the procedure and 24 h afterwards to visualize the residual intra-abdominal spread of the contrast medium. The video recordings and radiographs of all HSGs were discussed in a consensus meeting. Besides tubal patency and intra-abdominal spread of contrast medium, uterine malformations and intracavitary pathology were assessed. HSG was considered normal when both tubes were patent and contrast medium had spread normally after 24 h, or when only a uterine malformation or minor intracavitary pathology (both unlikely to be related to the patients’ fertility) was found. HSG was considered abnormal when both tubes were occluded and no spill of contrast medium was seen in the abdominal cavity after 24 h. HSG was considered inconclusive in all other cases (i.e. unilateral tubal occlusion, uncertain unilateral or bilateral tubal patency, high pressure needed to fill the tubes, pockets of contrast medium other than in the pouch of Douglas, or when the procedure had been abandoned).

Laparoscopy with tubal testing

In 355 patients, laparoscopy with tubal testing using Methylene Blue dye was performed. Tubal patency and the presence of peri-adnexal adhesions and endometriosis were assessed. For endometriosis, the 1996 revised American Society for Reproductive Medicine (rASRM) classification was used (The American Society for Reproductive Medicine, 1997). Laparoscopies were performed by gynaecologists who were not blinded for CAT and/or HSG results. For the present study, all laparoscopy reports were scored independently by two investigators (J.A.L. and J.L.H.E.) who were unaware of CAT and/or HSG results. In cases of disagreement, consensus was reached by consultation. On the basis of tubal patency and the presence of peri-adnexal adhesions and endometriosis, findings at laparoscopy were categorized into three levels according to the patient’s estimated spontaneous pregnancy chance: normal spontaneous pregnancy chance (subcategories: no abnormalities and no fertility-imparing factors), reduced spontaneous pregnancy chance and (nearly) absent spontaneous pregnancy chance, respectively. Subfertile women with reduced and (nearly) absent spontaneous pregnancy chances were considered together as a group having tubal pathology, whereas those with normal spontaneous pregnancy chances were considered not to have tubal pathology. This categorization was based on the Hull and Rutherford classification (Rutherford and Jenkins, 2002) and on our experts’ group opinion (J.A.L. and J.L.H.E.). Table I reflects the classification of the findings at laparoscopy.

No laparoscopy because of interval conception

No laparoscopy was performed in 205 patients, because they conceived either spontaneously or following treatment (other than IVF) before laparoscopy. Interval conception was used as a surrogate marker for the absence of tubal pathology, and therefore these 205 patients were categorized as having no tubal pathology (Table I), although it cannot be ruled out that minor degrees of tubal pathology may have been present. Patients who conceived by IVF were not included in this subgroup, because IVF-pregnancies are independent of tubal function.
No laparoscopy and no interval conception

In 82 patients, no laparoscopy was performed and no pregnancy occurred either spontaneously or after treatment (other than IVF) following HSG, because these patients decided to stop treatment, were advised to stop treatment or were referred for IVF. In this subgroup of patients, no reference standard was available and neither was a surrogate outcome.

Statistical methods

In the study population of 642 women, serum samples were missing from 167 patients, HSG was not performed in 218 patients and no laparoscopy or interval conception occurred in 82 women (Fig. 1). Exclusion of all patients with an incomplete data set would result in an inefficient reduction of the population size and might introduce bias if the excluded group were a non-randomly selected subsample from the entire population. Therefore, we used an alternative approach based on multiple imputations (Van Buuren et al., 1999; Altman and Bland, 2007; Horton and Kleinman, 2007). Five imputations were performed (A.G.H.K.), in which the missing data on CAT, hs-CRP and HSG were imputed as described extensively by Van Buuren et al., 1999. Imputed data were calculated by using all available data of the entire population. Each of the five completed data sets was analysed, and the results were integrated into a final result (C.M.J.G.L.) (Van Buuren et al., 1999; Horton and Kleinman, 2007).

In 82 patients, no reference standard was available (Fig. 1). Excluding this subgroup might also introduce selection bias. With a logistic regression model, we determined which subgroups were underrepresented and in all analyses, weights were used to compensate for this underrepresentation (Horton and Kleinman, 2007).

Screening strategies

Three screening strategies for tubal factor subfertility were evaluated comprising the test modalities: CAT, hs-CRP and HSG. In strategy A, HSG was performed. In strategy B, CAT and HSG were performed consecutively. In strategy C, CAT, hs-CRP and HSG were performed consecutively. All strategies were designed in a manner that tests were performed in the order of increasing invasiveness. Laparoscopy was used as reference standard for the presence or absence of tubal pathology, and the occurrence of a spontaneous pregnancy was used as a surrogate marker for the absence of tubal pathology. For all strategies, decision tables were constructed, in which all possible combinations of test results were listed (Glasziou and Hilden, 1986). Subsequently, the posterior probabilities of tubal pathology and 95% confidence intervals were calculated for all combinations using a logistic regression model. Furthermore, with this model, the significance of diagnostic coefficients was tested.

Results

Population characteristics

The study population consisted of 642 subfertile women who attended our Fertility Clinic and who consecutively underwent tubal testing by HSG and/or laparoscopy. In 71%, subfertility was primary and in 28%, it was secondary. In 1%, this information was not available. At intake, the median age was 31 years (range 19–41) and the median duration of subfertility was 17 months (range 0–162). Serum samples were obtained at the initial visit, and spare samples from 475 women were available for CAT and hs-CRP testing. Of these 475 women, CAT was negative in 88% and positive in 12% of patients. Hs-CRP was negative in 76% and positive in 24% of patients. HSG was performed in 424 women, of whom 66% had a normal HSG, 2% had an abnormal HSG and 32% had an inconclusive HSG. Laparoscopy was performed in 355 women, of whom 70% had no tubal pathology and 30% had tubal pathology. In 205 women, no laparoscopy was performed because of interval conception, and these patients were considered not to have tubal pathology. The median duration between initial visit and HSG was 4 months (range 0–59). The median duration between HSG and laparoscopy was 8 months (range 1–46). The median duration between initial visit and laparoscopy was 10 months (range 0–71). Figure 1 shows an overview of the obtained data (number of serum samples, HSG, laparoscopy and spontaneous pregnancies), the imputed data and the subgroup for which a weighted analysis was performed.

Table 1. Overview of the classification of tubal pathology and estimated spontaneous pregnancy chance, respectively, based on findings at laparoscopy (if available) or on interval conception.

<table>
<thead>
<tr>
<th>n</th>
<th>Estimated spontaneous pregnancy chance</th>
<th>Findings at laparoscopy, based on tubal patency and the presence of peri-adenal adhesions and endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tubal pathology (n = 455)</td>
<td>89 Normal (no abnormalities)</td>
<td>Patent tubes, no adhesions, no endometriosis</td>
</tr>
<tr>
<td></td>
<td>161 Normal (no fertility-impairing factors)</td>
<td>Patent tubes, no/any adhesions, no/Stage I endometriosis; unilateral tubal occlusion, no adhesions, no/Stage I endometriosis</td>
</tr>
<tr>
<td></td>
<td>205 Normal</td>
<td>No laparoscopy because of interval conception before laparoscopy (spontaneous or treatment-dependent conception, IVF-pregnancies not included)</td>
</tr>
<tr>
<td>Tubal pathology (n = 105)</td>
<td>58 Reduced</td>
<td>Patent tubes, any/extensive unilateral adhesions, no/Stage I/II/III endometriosis; patent tubes (high pressure needed), no/any/extensive adhesions, no/Stage I endometriosis; unilateral tubal occlusion with normal contralateral side, no/any/extensive unilateral adhesions, no/Stage I endometriosis</td>
</tr>
<tr>
<td></td>
<td>47 (Nearly absent)</td>
<td>Patent tubes, extensive bilateral adhesions, no/Stage I/II/III endometriosis; patent tubes (very high pressure needed), no/any/extensive adhesions, no/Stage I/II/III endometriosis; unilateral tubal occlusion with suboptimal contralateral side, no/any/extensive adhesions, no/Stage I/II/III endometriosis; bilateral tubal occlusion, no/any/extensive adhesions, no/Stage I/II/III endometriosis</td>
</tr>
<tr>
<td>Unknown (n = 82)</td>
<td>82 Unknown</td>
<td>No laparoscopy, no interval conception (active or passive discontinuation of fertility evaluation or referral for IVF)</td>
</tr>
</tbody>
</table>
**Screening strategies**

Figure 2 shows the results of screening strategy A (HSG). Figure 3 shows the results of screening strategy B (CAT → HSG). Figure 4 shows the results of screening strategy C (CAT → hs-CRP → HSG). The diagnostic performance of HSG, as expressed by the coefficients in the logistic regression model, was not statistically significant in all three strategies (P-values between 0.4 and 0.8). Moreover, the diagnostic performance of CAT in strategies B and C (P < 0.0001) and hs-CRP in strategy C (P < 0.003) were highly significant.

**Additional findings at HSG and laparoscopy**

**Uterine malformations and intracavitary pathology at HSG**

Uterine malformations were found in 2.4% of the HSGs (10/424): arcuate uterus (3), bicornuate uterus (3), unicornuate uterus (2), septate uterus (1) and T-shaped uterus (1). Intracavitary pathology was found or suspected in 2.1% of the HSGs (9/424): polyps (6), fibroids (2) and suspected intracavitary adhesions (1). In two patients with suspected polyps, and in the patient with suspected intracavitary adhesions, hysteroscopy was performed, in which no abnormalities were found. In the other six cases with intracavitary pathology, no hysteroscopy was performed and expectant management was observed.
Lesions suspect for endometriosis were found in 50% of all patients undergoing laparoscopy (176/355). Of these 176 patients, 88% had minimal peritoneal endometriosis without adhesions (compatible with rASRM Stage I), 5% had peritoneal endometriosis with adhesions (Stages II and III), 2% had ovarian endometriosis without adhesions (Stages I and II) and 6% had ovarian endometriosis with adhesions (Stages II and III). No Stage IV endometriosis was found at laparoscopy in our study population, which was according to Figure 3.

**Figure 3**: Strategy B: CAT → HSG.

**Endometriosis at laparoscopy**

Lesions suspect for endometriosis were found in 50% of all patients undergoing laparoscopy (176/355). Of these 176 patients, 88% had minimal peritoneal endometriosis without adhesions (compatible with rASRM Stage I), 5% had peritoneal endometriosis with adhesions (Stages II and III), 2% had ovarian endometriosis without adhesions (Stages I and II) and 6% had ovarian endometriosis with adhesions (Stages II and III). No Stage IV endometriosis was found at laparoscopy in our study population, which was according to Figure 4.

**Figure 4**: Strategy C: CAT → hs-CRP → HSG.
our expectation, because suspected severe endometriosis (based on history and findings at pelvic examination) was an exclusion criterion.

Discussion
In the present study, we assessed the clinical value of three different screening strategies for assessing the risk of tubal pathology in a population of subfertile women using: serological testing (CAT and hs-CRP) and HSG as screening methods, laparoscopy as a reference standard and the occurrence of a spontaneous pregnancy as a surrogate marker for the absence of tubal pathology. The aim was to develop a useful minimally invasive screening strategy which can be used in the diagnostic work-up of subfertile couples to estimate the risk of tubal pathology preceding laparoscopy. Estimating the cost-effectiveness of the different screening strategies or analysing cost-minimization of the screening strategies was beyond the scope of this study. Tubal pathology was defined as reduced or (nearly) absent spontaneous pregnancy chances based on tubal patency and the presence of peri-adnexal adhesions and endometriosis at laparoscopy. Patients with normal spontaneous pregnancy chances based on the laparoscopy reports and patients who conceived prior to laparoscopy were considered not to have tubal pathology (Table I). We have also re-analysed our data using a more strict definition of tubal pathology, in which the subgroup with reduced spontaneous pregnancy chances was categorized as having no tubal pathology. Obviously, the pre-test probability of tubal pathology was lower (8%), but the trends in post-test probabilities as noted in the present analysis remained unchanged (data not shown). In our opinion, the definition of tubal pathology which has been used in the present study [i.e. reduced or (nearly) absent spontaneous pregnancy chances] is applicable to the average subfertile population. The more strict definition may be used in a clinical setting in which only a very high suspicion of tubal pathology, based on the results of a screening strategy, may alter clinical decision-making (e.g. in the case of limited access to operating facilities).

Strategy A: HSG
According to the fertility-guideline of the National Institute for Clinical Excellence (NICE, 2004), HSG should be offered to women who are not known to have co-morbidities (such as a history of pelvic inflammatory disease, previous ectopic pregnancy or endometriosis) to screen for tubal pathology. In women who are assumed to have co-morbidities, laparoscopy should be offered instead of HSG. Strategy A corresponds with the screening method proposed by NICE, in which only women at low risk for tubal pathology (based on the medical history) undergo HSG.

In our study population, consisting of women without known co-morbidities, the pre-test probability of tubal pathology was 19% and the post-HSG probabilities ranged from 16% to 26% (95% CI 0–58), depending on the HSG result. It should be noted that the risk of tubal pathology did not differ between women with a normal HSG versus an inconclusive HSG (e.g. unilateral tubal occlusion, uncertain unilateral or bilateral tubal patency, high pressure needed to fill the tubes and pockets of contrast medium other than in the pouch of Douglas), indicating that inconclusive HSGs can be categorized as normal. These results are in accordance with a study of Mol et al. (1999), who conclude that fertility prospects in women with unilateral tubal occlusion at HSG are only slightly lower when compared with women with bilateral tubal patency at HSG. The risk of tubal pathology in the case of a normal or inconclusive HSG is slightly lower when compared with findings of previous studies, which state that ~20–25% of women with normal HSG findings have tubal pathology caused by adhesions or endometriosis (Henig et al., 1991; Tanahatoe et al., 2003), which often remain undetected at HSG (Swart et al., 1995). From our data, it can be concluded that in low pre-test risk women, a normal or inconclusive HSG is of little additional value in discerning women with a high versus low post-HSG risk of tubal pathology. No conclusion can be drawn in the case of an abnormal HSG, owing to the wide 95% confidence interval of post-HSG risk of tubal pathology (0–58), although the prevalence of abnormal HSGs in our low-risk population is only 2% (9/424).

Strategy B: CAT→HSG
CAT has been introduced in the fertility evaluation as a screening test to estimate the risk of tubal pathology, because an association exists between the presence of C. trachomatis IgG antibodies in serum and tubal pathology (Punnonen et al., 1979). The value of CAT in assessing the risk of tubal pathology is at least comparable with HSG (Dabekausen et al., 1994; Perquin et al., 2007). The advantage of CAT is that it is a simple, inexpensive and minimally inconvenient test method, which makes CAT recommended in subfertile women as the first screening test for tubal pathology after medical history taking (Dabekausen et al., 1994; Mol et al., 1997, 2001; Veenemans and Van der Linden, 2002; Coppus et al., 2007). Strategy B reflects the use of CAT prior to HSG (in the order of increasing invasiveness). Our data show that CAT accurately discerns patients with a high versus low risk of tubal pathology [post-test probability 14% (95% CI 4–23) in CAT-negative patients and 53% (95% CI 16–90) in CAT-positive patients]. Our present findings on the predictive value of CAT for tubal pathology are comparable with those reported by others (as summarized by Den Hartog et al., 2006). Performing HSG after CAT does not change the probability of tubal pathology significantly. In CAT-negative women (post-CAT probability of tubal pathology 14%), the post-HSG probabilities ranged between 13% and 17% (95% CI 0–41), whereas in CAT-positive women (post-CAT probability of tubal pathology 53%), the post-HSG probabilities ranged between 53% and 60% (95% CI 17–100). From these results, it can be concluded that CAT, as a first screening test to estimate the risk of tubal pathology in the fertility work-up, is more accurate in comparison with HSG (strategy A). In both CAT-negative and CAT-positive women, HSG following CAT has no significant additional value in estimating the risk of tubal pathology. Furthermore, it should be taken into account that HSG in CAT-positive women holds a 10% risk of post-HSG complications (e.g. fever and infection) in
women with tubal pathology (Forsey et al., 1990). Therefore, the value of HSG following CAT in screening for tubal factor subfertility should be critically reappraised: the post-HSG risk of tubal pathology in CAT-negative and CAT-positive women remains invariably low and high, respectively; HSG is relatively contra-indicated in CAT-positive women because of increased post-infectious morbidity and CAT-positive women may benefit from early laparoscopy in order to provide a definitive diagnosis without delay.

**Strategy C: CAT → hs-CRP → HSG**

Because persistence of *C. trachomatis* infections is an important risk factor for tubal pathology, the additional value of serological markers of persistence has been evaluated previously (Den Hartog et al., 2005). Combining CAT (as a marker of a previous *C. trachomatis* infection) and hs-CRP (as a marker of persistence) has been found a valuable test set to identify women at highest risk of tubal pathology (Den Hartog et al., 2005). Strategy C reflects the use of hs-CRP following CAT and preceding HSG. Because hs-CRP is a general marker of inflammation, it is considered of importance in CAT-positive women only, in whom it may discern women with a high risk of persistence and tubal pathology (both CAT and hs-CRP positive) from those with a lower risk of persistence and tubal pathology (CAT positive and hs-CRP negative). Our results confirmed that the value of hs-CRP in assessing the risk of tubal pathology in CAT-negative women is limited. In CAT-positive women [post-CAT probability of tubal pathology 53% (95% CI 16–90)], the post-hs-CRP probabilities were 45% (95% CI 32–58) in hs-CRP-negative women and 66% (95% CI 54–79) in hs-CRP-positive women, respectively. After adding HSG as a third test, the probability of tubal pathology does not change significantly [44–71% (95% CI 8–100)]. It can be concluded that hs-CRP, which is simple, inexpensive and minimally inconvenient to patients and has previously been shown to improve the predictive value of CAT significantly (Den Hartog et al., 2005), seems of additional value in estimating the risk of tubal pathology in CAT-positive subfertile women only. As discussed in strategy B, HSG has little additional value in assessing the risk of tubal pathology in CAT-negative women, and is relatively contra-indicated in CAT-positive women.

**Limitations of the study**

Our study has several limitations. First, serum samples from 167 patients (mainly those who visited our clinic in the early 1990s) were missing. Although CAT had been performed as a part of their fertility evaluation, no spare samples were available for the present study. The original CAT results were obtained by a different CAT test, which has later been shown to be inferior to the CAT test used in the present study (Land et al., 2003), and therefore it was decided not to use the results of the original CAT. Missing data on CAT and hs-CRP were replaced by plausible values by using multiple imputations (Van Buuren et al., 1999; Altman and Bland, 2007; Horton and Kleinman, 2007). An important limitation of our study is verification bias, because the CAT result in the fertility evaluation determined whether HSG or laparoscopy was performed. In our clinic, HSG is not performed in CAT-positive women owing to the 10% risk of febrile and infectious morbidity reported in women with tubal pathology (Forsey et al., 1990). Therefore, abnormal HSGs are rare (9/424 = 2%), causing wide ranges in the 95% confidence intervals. We have used multiple imputations to fill in the missing data of HSG, in order to minimize the influence of verification bias (Van Buuren et al., 1999, Altman and Bland, 2007, Horton and Kleinman, 2007). This verification bias is difficult to avoid, unless one is willing to perform HSG in all subfertile women, thereby accepting its potential harmful effects, and unless one is prepared to perform HSG and laparoscopy in all women, preferably even on the same day. Another contributor to verification bias is that only patients who underwent tubal testing (HSG and/or laparoscopy) were included. Previously, we have calculated that 70–80% of all subfertile women who start a fertility work-up will not undergo tubal testing, because of spontaneous pregnancies, immediate referral for IVF or drop-out (Fiddelers et al., 2005). The risk of tubal pathology will therefore be overestimated in our study. Finally, we are aware that nowadays other methods for evaluating tubal function [such as hysterosalpingo (contrast) sonography and transvaginal hydrolaparoscopy] are used, but we have limited our study to HSG and laparoscopy because these well-evaluated tests are performed on a routine basis in most clinics.

**Omitting HSG?**

It may be questioned whether HSG should still be performed in the fertility investigation, as our results indicate that HSG has limited value in the risk assessment of tubal pathology and is relatively contra-indicated in CAT-positive women. Although HSG may provide additional information on uterine malformations and intracavitary pathology, their prevalence is low (in our study 2.4% and 2.1%, respectively, which is comparable with prevalences found in the literature) (Varasteh et al., 1999, Grimbizis et al., 2001). The majority of these abnormalities can also be diagnosed by ultrasonography (which in most clinics is performed routinely in all subfertile patients), their presumed effect on fertility is debatable, and the effectiveness of treatment of uterine abnormalities on improving pregnancy rates has not been established (NICE, 2004). However, HSG also has advantages; therefore, omitting HSG in CAT-negative women deserves careful consideration. The first advantage of HSG is that it has a high specificity (Swart et al., 1995), i.e. HSG is accurate in confirming the absence of tubal pathology. Furthermore, tubal flushing using oil-soluble contrast medium has a positive effect on pregnancy rates (Luttjeboer et al., 2007). As a consequence, omitting HSG using oil-soluble contrast medium may result in lower pregnancy rates. So far, this positive effect on pregnancy rates has not been found when water-soluble contrast medium was used (Perquin et al., 2006). On the basis of these considerations, the role of HSG in the fertility evaluation of low-risk patients (i.e. CAT-negative women) deserves critical reappraisal. In CAT-positive high-risk patients, HSG should be omitted because of the 10% risk of post-HSG complications in the absence of additional value in risk assessment of tubal pathology.
**Omitting laparoscopy?**

Although laparoscopy is the reference standard in diagnosing tubal pathology, it may be questioned whether it should be performed routinely in all patients. Laparoscopy is still considered a useful test in women with known co-morbidities which may have compromised tubal function, such as a history of pelvic inflammatory disease (NICE, 2004). In patients with an uneventful medical history, it has been suggested that laparoscopy can be omitted after a normal HSG (Fatum et al., 2002; Lavy et al., 2004), although prevalence figures for tubal pathology of ~20–25% have been reported in women with normal HSGs (Henig et al., 1991; Tanahatoe et al., 2003). In our study, the prevalence of tubal pathology in CAT-negative women ranged from 10% to 22% in patients with a normal HSG and from 12% to 25% in patients with an inconclusive HSG. Omitting laparoscopy in patients with a very low (or very high) risk of tubal pathology, based on the results of screening tests, implicates that potential other causes of subfertility, such as endometriosis, remain undiagnosed and hence untreated. In our study, half of all patients undergoing laparoscopy had endometriosis, but this was limited to Stages I and II disease in over 90% of them. It is subject of discussion whether Stages I and II endometriosis reduce fertility and should therefore be treated. A meta-analysis of two randomized controlled trials showed that subfertile women with Stages I and II endometriosis benefit at least temporarily from laparoscopic surgery (Jacobson et al., 2002; Kennedy et al., 2005), although the number needed to treat is 24 (i.e. 24 subfertile women need to undergo laparoscopy in order to identify and treat 12 women with Stages I and II endometriosis and to achieve one additional pregnancy) (ESHRE Capri Workshop Group, 2004). The decision whether to perform a laparoscopy in patients with an uneventful medical history and normal pelvic examination therefore depends on how certain one wishes to be in finding fertility-impairing factors, and on the value attributed to treating Stages I and II endometriosis. Furthermore, it remains difficult to establish the precise position and timing of laparoscopy in subfertile women due to a lack of randomized controlled studies (Bosteels et al., 2007).

Practically, in couples with an otherwise unexplained subfertility, a negative CAT and a normal or inconclusive HSG, laparoscopy may be substituted by several cycles of combined gonadotrophins and intrauterine insemination, and subsequently IVF in those who fail to achieve a pregnancy (Fatum et al., 2002). This strategy limits the number of unnecessary laparoscopies and does not introduce a large delay in treatment in the small proportion of women who would have been diagnosed with tubal pathology at laparoscopy, especially now that IVF is rapidly replacing reconstructive microsurgery as the treatment of choice in these patients. On the basis of our data, the clinical consequences of omitting laparoscopy in these low-risk patients seem limited, because after appropriate screening, little residual unexpected (and treatable) pathology is found. In CAT-positive women, laparoscopy remains justified in order to prevent unnecessary delay before going for IVF in those with abnormal findings and in order to prevent going for IVF prematurely in those in whom less demanding treatment options might still offer acceptable pregnancy chances. Furthermore, the subgroup of women with bilateral tubal occlusion at HSG also clearly benefits from laparoscopy with tubal testing, because laparoscopy shows not more than unilateral tubal pathology in the majority of these women (Mol et al., 1999; Bosteels et al., 2007), as was confirmed in our study. In this subgroup, immediate IVF can thus be avoided in the majority of women by excluding bilateral tubal occlusion at laparoscopy.

**Summary and conclusion**

We evaluated three screening strategies, comprising serological testing (CAT and hs-CRP) and HSG, for assessing the risk of tubal pathology [i.e. reduced or (nearly) absent spontaneous pregnancy chances] in a population of subfertile women. Laparoscopy was used as reference standard for detecting tubal pathology, and the occurrence of a spontaneous pregnancy was used as a surrogate marker for the absence of tubal pathology. Despite several limitations of the study, the results of the present study may be used to optimize the risk assessment for tubal factor subfertility. CAT is the most valuable test to screen for tubal pathology, because it accurately discerns low-risk patients (negative CAT) from high-risk patients (positive CAT). On the basis of biological grounds, hs-CRP may be applied in CAT-positive patients only. To assess the additional role of hs-CRP more accurately, further studies are needed. HSG has little additional value in estimating the risk of tubal pathology in low-risk and high-risk patients. In low-risk patients, HSG may be performed to confirm the absence of tubal pathology and to allow the patient to benefit from the positive perturbation effect of tubal flushing with oil-soluble contrast medium on pregnancy rates. In high-risk patients, HSG is relatively contra-indicated owing to the 10% risk of post-HSG febrile and infectious morbidity in patients in whom tubal pathology is confirmed. In these high-risk patients and in women with bilateral tubal occlusion at HSG, laparoscopy is justified to provide a definite diagnosis. The role of laparoscopy in low-risk patients deserves critical reconsideration. The disadvantage of omitting laparoscopy would be that non-C. trachomatis-associated tubal pathology such as endometriosis (which was present in half of the women in our study population undergoing laparoscopy) remains undiagnosed and untreated. However, at least 90% of women with endometriosis at laparoscopy were classified as having Stages I or II disease, of which the lasting benefit of treatment is still a matter of debate. The consequences of omitting laparoscopy in these women may be of limited clinical significance, especially since eventually all those failing to conceive will be referred for IVF.

On the basis of the findings of the present observational study, we propose CAT as the first screening test for tubal pathology in subfertile women. In CAT-negative women, HSG may be performed because of its high specificity and, when using oil-soluble contrast medium, its fertility-enhancing effect. In CAT-positive women, hs-CRP is promising, although more studies are needed to further corroborate the value of
hs-CRP in this respect. HSG has no additional diagnostic value and is relatively contra-indicated in CAT-positive women. The precise position and timing of laparoscopy deserves critical reappraisal and depends on the risk of tubal pathology, as assessed by screening tests. In CAT-negative patients and in patients with normal or inconclusive HSGs, laparoscopy may be omitted. In CAT-positive women and in women with abnormal HSGs, laparoscopy remains justified.

References


