



Endometriosis screening in patients attending an IVF clinic: a proof-of-concept retrospective cohort study

Loren Mear, Charles Pineau, François Vialard, Juan-Felipe Velez de La Calle

► To cite this version:

Loren Mear, Charles Pineau, François Vialard, Juan-Felipe Velez de La Calle. Endometriosis screening in patients attending an IVF clinic: a proof-of-concept retrospective cohort study. *Human Fertility*, 2022, 25 (2), pp.313-322. 10.1080/14647273.2020.1795731 . hal-02928422

HAL Id: hal-02928422

<https://hal.science/hal-02928422>

Submitted on 26 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

ORIGINAL ARTICLE

**Endometriosis screening in patients attending an IVF clinic: a proof-of-concept
retrospective cohort study.**

Loren Méar^{a,b,e}, Charles Pineau^{a,e}, François Vialard^{b,c}, Juan-Felipe Velez de la Calle^d

^aUniv Rennes, INSERM, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes cedex, France; ^bGIG-EA7404, Université de Versailles Saint-Quentin-en-Yvelines – Paris Saclay, UFR Sciences de la Santé-Simone Veil, F-78180 Montigny-le Bretonneux, France; ^cDepartment of Biology of Reproduction, Cytogenetics and Genetics, Centre Hospitalier de Poissy-Saint Germain, CS 73082, F-78303 Poissy, France; ^dIVF Unit, Clinique Pasteur, F-29200 Brest, France; ^eProtim, Univ Rennes, F-35042 Rennes cedex, France.

CONTACT: Loren Méar, GIG-EA7404, Université de Versailles Saint-Quentin-en-Yvelines – Paris Saclay, UFR Sciences de la Santé-Simone Veil, F-78180 Montigny-le Bretonneux, France E-mail: loren.mear@gmail.com.

ABSTRACT

Given that (i) endometriosis affects approximately 40% of women diagnosed with fertility problems and (ii) this condition may be an underestimated cause of idiopathic infertility, it is essential to identify high-risk patients for laparoscopic screening and reduce the diagnostic delay. We performed a retrospective analysis of 312 women (208 diagnosed with endometriosis and 104 controls) admitted to an *in vitro* fertilization (IVF) unit in the city of Brest (France) between June 2007 and July 2014. As part of the women's infertility treatment, levels of cancer antigen 125 (CA-125) were assayed in blood samples collected on the day of oocyte retrieval. Surplus serum was used to set up a new sperm agglutination test. It was observed that sperm agglutination was significantly correlated with endometriosis and CA-125 levels ($p < 0.01$ for both). By building a decision tree, we identified a subpopulation of patients with low CA-125 levels and a high risk of endometriosis. This proof-of-concept study constitutes a first step towards a high-quality, controlled, multi-centre trial. If our preliminary results are confirmed, the decision tree should improve the medical care given to women in IVF programmes by identifying potential endometriosis sufferers for laparoscopic examination and enabling them to be counselled about precautionary measures.

Keywords: Endometriosis screening; IVF; high-risk population

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Endometriosis is a gynaecological disease characterized by the presence and development of functional endometrial tissue outside the uterine cavity (Giudice & Kao, 2004). The disease is frequent, since it affects 10% of women of reproductive age (i.e. from puberty to menopause) and nearly 50% of infertile women (regardless of whether or not they experience pelvic pain) (Giudice & Kao, 2004).

The lack of specific symptoms means that endometriosis is difficult and time-consuming to diagnose. Indeed, it may take over 5 years to establish a firm diagnosis and to provide the patient with appropriate treatment. The diagnostic delay for endometriosis is 8 years in the UK (Ballard et al., 2006) and 10.5 years in Austria and Germany (Hudelist et al., 2012). We currently lack a reliable blood-based screening test for endometriosis (May et al., 2010; Vinatier et al., 2001): those developed to date have a low level of sensitivity or are poorly specific (Bedaiwy & Falcone, 2004; Nisenblat, Bossuyt, et al., 2016). Radiological examinations (e.g. MRI and ultrasound) enable specialists to detect most large endometriotic lesions. Laparoscopy is often considered to be the gold standard for the diagnosis of endometriosis; this invasive surgical procedure not only enables the collection of a biopsy for subsequent histological analysis but also allows the spread of the disease to be visually assessed (Fassbender et al., 2015; Rogers et al., 2017; Spaczynski & Duleba, 2003). Although accurate ultrasound monitoring might be enough for suspecting endometriosis in an infertile population (Guerriero et al., 2018), the diagnosis of endometriosis is still challenging in a proportion of these patients.

An association between endometriosis and elevated serum levels of cancer antigen 125 (CA-125) was described in the mid-1980s (Mol et al., 1998). CA-125 is a mucin glycoprotein encoded by the *MUC16* gene, and was initially studied as a serum

marker of the progression and presence of ovarian cancer (Fassbender, et al., 2015; Gupta & Lis, 2009; Nisenblat, Prentice, et al., 2016). This mucin has also been extensively assessed as a potential biomarker for endometriosis (May et al., 2010; Nisenblat, Bossuyt, et al., 2016). Many studies have shown that the serum CA-125 level is correlated with the severity of endometriosis in general (May et al., 2010) and of ovarian endometrioma in particular. Although elevated CA-125 levels may be associated with other gynaecologic disorders (especially ovarian tumors) (Bedaiwy & Falcone, 2004; Fassbender et al., 2015), the concentration can vary during the menstrual cycle (May et al., 2010) and might have less diagnostic value in the early stages of endometriosis (Mol et al., 1998). In a meta-analysis, CA-125 had a sensitivity of 52% and a specificity of 93%, and the marker's diagnostic performance did not depend on the stage of endometriosis (Hirsch, Duffy, Davis et al., 2016). Although CA-125's diagnostic sensitivity and specificity vary according to the choice of the threshold, none of the published values are sufficiently reliable for use in a diagnostic test (Nisenblat, Bossuyt, et al., 2016).

Several studies have highlighted the influence of endometriosis on fertility, with a negative impact on oocyte quality and embryonic development, and an increase in the risk of miscarriage (Stilley et al., 2012; Tanbo & Fedorcsak, 2017). Since the 1990s, several observations have emphasized the negative effect of endometriosis on spermatozoa, such as: (i) lower sperm binding to the zona pellucida (Coddington et al., 1992); (ii) reduced binding of sperm to endosalpinx of women with endometriosis (Reeve et al., 2005) (iii) the presence of elevated levels of inflammatory mediators in peritoneal fluid from women suffering from endometriosis, which may be responsible for damage to sperm DNA (Mansour et al., 2009); and (iv) the elevated levels of preinterleukin-6 in peritoneal fluid, which may also inhibit sperm motility (Yoshida et

1
2
3 94 al., 2004). More recently, it has been shown that peritoneal fluid from women suffering
4
5 95 from endometriosis reduces sperm motility and quality (Sáez-Espinosa et al., 2019). As
6
7 96 mentioned above, it has been estimated that 25 to 50% of infertile women have
8
9 97 endometriosis (Bulletti et al., 2010) and that women with endometriosis have an
10
11 98 increased risk of infertility: in one study, the odds ratio (95% confidence interval (CI))
12
13 99 was 6.2 (5.4–7.1) (Ballard et al., 2008). However, the putative underlying link between
14
15 100 endometriosis and infertility has yet to be characterized and is subject to debate
16
17 101 (Somigliana et al., 2017).

21 102 The present study was prompted by a number of anecdotal but recurrent
22
23 103 observations made in an *in vitro* fertilization (IVF) unit in Brest (France) for
24
25 104 conventional IVF procedures (i.e. no intracytoplasmic sperm injection) involving
26
27 105 patients with endometriosis. The day after IVF, we found that the remaining
28
29 106 spermatozoa in the culture wells tended to agglutinate, whereas non-treated
30
31 107 spermatozoa from the same donor did not. Given that sperm are incubated with the
32
33 108 cumulus oophorus during an IVF procedure, we hypothesized that (i) this agglutination
34
35 109 phenomenon might be due to the presence of a specific protein in follicular fluid from
36
37 110 women with endometriosis, and (ii) the specific protein might also be present in the
38
39 111 woman's blood serum. On the basis of these anecdotal observations and the literature
40
41 112 data, we attempted to (i) reproduce this sperm agglutination phenomenon in the
42
43 113 laboratory, and (ii) determine whether the agglutination was specifically related to
44
45 114 contact with biological fluids from IVF patients with endometriosis.

51 115
52
53 116 **Material and methods**

56 117 *Patients*

58 118 Patients attending the IVF unit at the Clinique Pasteur (Brest, France) from June 2007 to

July 2014 were included in the study. The sole inclusion criterion was admission to an IVF programme. Two groups of participants were defined: (i) women with laparoscopically and histologically confirmed endometriosis (hereafter referred to as the endometriosis group), and (ii) women without a documented history of endometriosis (the control group). For ethical reasons, the absence of endometriosis was not confirmed laparoscopically in the control participants; there was no justification for an invasive surgical procedure in these patients.

All women received standard treatment for IVF, namely the administration of a gonadotropin-releasing hormone (GnRH) antagonist and gonadotropin. None of the women received a GnRH agonist before or during the IVF programme. A blood sample was always collected (for hormone assays) on the day of oocyte retrieval. The remaining blood (typically considered as clinical waste) was used for the present study.

Capacitated sperm samples from 30 male patients requiring a conventional semen analysis were selected for the agglutination tests. The inclusion criteria were as follows: (i) the absence of spermatozoon aggregation or agglutination; (ii) a clearly negative mixed antiglobulin reaction assay (the SpermMAR Test IgG and SpermMAR Test IgA, FertiPro N.V., Beernem, Belgium); (iii) a normal sperm count (as defined in the World Health Organization's Laboratory Manual for the Examination and Processing of Human Semen, fifth edition, i.e. $>39 \times 10^6$ per ejaculate); (iv) more than 75% of progressively motile spermatozoa; and (v) the absence of leukocytes. When all the standard laboratory analyses had been completed, the remaining sperm (again typically considered as clinical waste) was used in the agglutination test. The sperm samples had been collected at the end of the study inclusion period (i.e. from July to September 2014).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

143 The blood and sperm samples were collected as part of the routine procedure for
144 patients undergoing IVF, and no additional procedures were required. All patients
145 provided their prior, written, informed consent to participation prior to sampling. The
146 study data were anonymized before the analysis. The study was approved by the
147 Clinique Pasteur’s institutional review board (Brest, France; reference: 21CHP).

148

149 *Sample preparation and processing*

150 Sera were obtained by centrifugation of the clotted blood sample at 1.5 x g for 10
151 minutes. An aliquot of serum (of at least 200 µL) was used for the CA-125 assay,
152 whereas the remaining volume was frozen at -20°C until required for the sperm
153 agglutination test.

154 Serum CA-125 levels were measured using a Roche Elecsys® CA 125 II assay
155 on a Roche Cobas® system (Roche Diagnostics, Indianapolis, IN, USA), according to
156 the manufacturer’s instructions.

157 After thawing, the sera were incubated at 56°C for one hour so that the
158 complement components were inactivated. For each patient, two dilutions (1:2 and 1:4)
159 of complement-depleted serum were tested, in order to avoid false negatives (caused by
160 a zone phenomenon) as much as possible. Complement-depleted sera were then diluted
161 in pre-warmed (37°C) FertiCult® medium (JCD Laboratoires, La Mulatière, France).
162 Next, a 50 µL drop of diluted serum was mixed with a 2.5 µL of suspended post-
163 capacitation sperm (corresponding to approximately 65.10³ spermatozoa) in a Petri dish.
164 The droplets were completely covered with oil for embryo culture (Irvine Scientific;
165 catalog number: 9305), and the closed Petri dishes were incubated overnight at 37°C in
166 a humidified 5% CO₂ atmosphere. On the following day, each droplet in the Petri dish
167 was examined under a Diaphot 300 microscope (Nikon, Japan). Based on the number of

agglutinates per view of field and the WHO guidelines, the agglutination was classified semi-quantitatively into three groups: (i) no agglutinate (referred to as “–”); (ii) a low proportion of agglutinated sperm (less than 4 agglutinates per field; “+”); or (iii) a high proportion of agglutinated sperm (4 or more agglutinates per field; “++”) (Fig 1 a, b, c). This classification did not take account of the number of spermatozoa per agglutinate or the way in which the motile spermatozoa stuck to each other (i.e. agglutination specifically). The experimental workflow is detailed in Fig 1d. The experiments were performed in 2016. Negative controls were always performed by omitting the complement-depleted serum.

Statistical analysis

The threshold for statistical significance was set to $p < 0.01$. Standard non-parametric tests were used to compare cases with controls. Since the quantitative data (age, and the serum CA-125 level) were not normally distributed (as confirmed by Shapiro’s test), a Mann-Whitney or a Wilcoxon test was applied. However, a t-test was performed to confirm the previous results. Quantitative variables were transformed into qualitative variables for subsequent analysis; thus, we created two age classes (< 35 and ≥ 35 years) and two categories for the CA-125 level (< 30 U/mL vs. ≥ 30 U/mL). Our choice of the 30 U/mL cut-off for CA-125 was based on the report by Hirsch, Duffy, Deguara, et al. (2016). Associations between qualitative variables (the results of the sperm agglutination test, age, and the serum CA-125 level) and endometriosis (cases vs. controls) were assessed using a chi-squared test.

Since two of the variables were quantitative (i.e. the CA-125 concentration, and age), we decided to replace them with the corresponding qualitative variables; this simplified the decision tree and made it more representative and reliable.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

The selected variables were included in a multivariate analysis, and the ORs (95%CI) were obtained by logistic regression. In order to validate our model, random sampling was used to create two datasets: a training set (corresponding to 70% of the entire dataset) and a test set (corresponding to 30%). We then applied the model derived from the training set to the test set, and calculated the sensitivity and the specificity. Using Breiman’s Classification and Regression Tree (CART) method (Breiman et al., 1984), we generated a decision tree on the basis of the final model and the training and test subsets.

In order to validate the decision tree, we performed a random forest analysis for significant variables. The term “random forest” encompasses an ensemble of learning methods that generate a multitude of decision trees, with bootstrapping (i.e. a “random with replacement” sampling method). Each tree was trained on 63% of the data. The remaining 37% were used to calculate the misclassification rate (i.e. the “out of bag” (OOB) error rate). An overall OOB error rate was obtained by aggregating the OOB error rate for each tree. Here, we used a random-forest-type classification.

We obtained 200 trees with random bootstraps from the entire original dataset. Lastly, the decision tree’s quality was determined in terms of its specificity, sensitivity, positive predictive value, and negative predictive value.

We used the methods implemented in R (R version 3.3.0, Comprehensive R Archive Network, CRAN), and the following R packages: MASS (Venables & Ripley, 2002), rpart (Therneau et al., 2015), randomForest (Liaw & Wiener, 2002), caret (Kuhn, 2016), partykit (Hothorn & Zeileis, (2015). partykit: A Modular Toolkit for Recursive Partytioning in R. Journal of Machine Learning Research, 16, 3905-3909. Retrieved from <http://jmlr.org/papers/v16/hothorn15a.html>, 2015), ggplot2 (Wickham, 2009), ROCR (Sing et al., 2005) and wesanderson (Ram et al., 2015).

218

219 **Results**220 *Characteristics of the study population*

221 A total of 312 women (208 with endometriosis, and 104 controls) were included in the
 222 study. The endometriosis and control groups did not differ significantly with regard to
 223 age (mean \pm SD (range) age: 34.1 ± 4.3 (25-43) and 35.2 ± 4.6 (25-43), respectively;
 224 $p > 0.01$ in both a Wilcoxon test and a confirmatory t-test) (S1 Fig). The serum CA-125
 225 level was significantly higher ($p < 0.001$) in the endometriosis group (mean (range): 43.4
 226 ± 43.8 U/mL (4.3-283.7) than in the control group (21.2 ± 12.6 U/mL (5.9-70.5);
 227 $p < 0.001$ in a Wilcoxon test). The incidences of high and moderate sperm agglutination
 228 were significantly higher ($p < 0.0005$) among patients suffering from endometriosis
 229 (72.26% with a 1:4 dilution, compared with 57.69% among controls). The qualitative
 230 variables are described in Table 1, and the two random subsets (the training and test
 231 sets) used to build the CART are described in Table 2.

232

233 *Identification of patient groups with a high risk of endometriosis*

234 The chi-squared test evidenced a statistically significant association between the serum
 235 CA-125 level and endometriosis. The results were significant for the agglutination test
 236 with a 1:2 dilution and with a 1:4 dilution (Table 1).

237 A decision tree was derived from a logistic regression analysis of the training set
 238 (Table 3 and Figure 2). The proportion of agglutination (for the 1:4 dilution) and the
 239 serum CA-125 concentration appeared to be of relevance for discriminating between the
 240 endometriosis and control groups. The decision tree identified five subgroups of
 241 patients (nodes 4, 6, 7, 8 and 9) with an increased risk of endometriosis; it is noteworthy
 242 that two of the high-risk populations (nodes 8 and 9 in Fig 2) could be identified with

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

two parameters only: the CA-125 level (with the usual cut-off of 30 U/mL) and the level of agglutination. Node 8 is interesting because it corresponds to a population with a low CA-125 level and a high (>70%) risk of endometriosis. This model had an accuracy (95% CI) of 0.74 (0.6408 – 0.8271), a sensitivity of 51%, a specificity of 85%, a positive predictive value of 64%, and a negative predictive value of 78% (Figure 2).

Two hundred trees were obtained: the OOB error rate was stable from the 50th tree onwards. Three variables were tested, each split and the estimated OOB error rate was 23.72% (S2 Figure B). A variable importance plot highlighted the importance of both the agglutination level and the CA-125 concentration in the prediction and classification models (Supplemental Figure 2A). The “agglutination level” variable was the most important for the two criteria (i.e. the accuracy and the Gini index) (S2 Figure B) used to evaluate the splits in the trees. Our random forest model had an accuracy (95%CI) of 0.76 (0.7116–0.8089), a sensitivity of 52%, a specificity of 88%, a positive predictive value of 69%, and a negative predictive value of 79%.

Discussion

In the present study, we developed a novel method for evaluating the probability of endometriosis in women in an IVF programme. The decision tree associated with the test was based on three main parameters: the agglutination level (for a 1:4 sperm dilution), the serum CA-125 level, and age. Our results confirmed that the primary observation of sperm agglutination in the culture well was correlated with the presence of endometriosis in the patient (Table 1), and emphasized the potential impact of endometriosis on spermatozoa. Indeed, a chi-squared test showed a significant association between the agglutination test result and endometriosis in the study population (p<0.01). The association between the serum CA-125 level and the presence

of endometriosis was also significant ($p < 0.01$) (Table 1). However, the main study objective of was to identify individuals at a high risk of endometriosis for women attending an IVF clinic, rather than to develop a new diagnostic test *per se*.

Here, we described a simple model based on logistic regression with the agglutination level, the serum CA-125 level (as a qualitative variable), and age (as a qualitative variable) as predictors, in order to discriminate between control patients and those suffering from endometriosis. We evaluated the model's accuracy in a random forest analysis with 200 trees. Our results indicate that this model is highly accurate (76%) for detecting endometriosis among sub-fertile women. Nevertheless, our model is based only on qualitative data and so provides limited sensitivity (52% only).

At present, there are no reliable biomarkers for endometriosis screening. Here, we demonstrated that the serum CA-125 assay is of value when a cut-off of 30 U/mL is applied (Kafali et al., 2004). Furthermore, given the risks associate with surgery, laparoscopy is being performed less frequently in patients with idiopathic infertility (Practice Committee of the American Society for Reproductive Medicine, 2015); this complicates the diagnosis of endometriosis in women undergoing IVF procedures. Therefore, a simple, inexpensive, non-invasive screen for women in IVF programmes would be a valuable option for restricting surgery to individuals with a high risk of endometriosis. Although our model cannot rule out the possibility of endometriosis, it might prevent unnecessary surgery and improve the management of endometriosis in sub-fertile women.

The present study was designed to describe the link between endometriosis on one hand and spermatozoa agglutination in culture wells containing oocytes on the other. The combination of this agglutination test with a serum CA-125 assay is a relevant indicator of the need for diagnostic laparoscopy in women undergoing an IVF

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

procedure. If endometriosis is diagnosed, appropriate care and treatment should improve the live birth and pregnancy rates (Blanc & von Theobald, 2017; Somigliana et al., 2017). The value of surgical endometriosis treatment in improving fertility and the pregnancy rate is subject to debate because the beneficial effects may have been overestimated (Somigliana & Garcia-Velasco, 2015; Somigliana et al., 2017); nevertheless, surgical treatment appears to be very effective for deep endometriosis (Roman et al., 2018).

The present study had a number of limitations. Firstly, only women undergoing an IVF procedure were included. All of the participants had been treated with a GnRH antagonist and gonadotropin. Hence, our results cannot necessarily be extrapolated to populations of women not treated with these hormones.

Secondly, the control population (with no history of endometriosis) was a major source of bias: the control participants had not systematically undergone a laparoscopic assessment to confirm the absence of endometriosis. However, given that these participants received high-quality medical care during the IVF procedure (especially with regard to gynaecological disease), we expect that the incidence of endometriosis in the control was probably similar to (or even lower than) that observed in the general population (i.e. 6 to 10%). Furthermore, it is important to bear in mind that the prevalence of endometriosis is higher (as much as 75%) in symptomatic populations (those with chronic pelvic pain, for example) (Hirsch, Duffy, Deguara, et al., 2016), and especially in infertile populations; in one study, at least 14% of women in sub-fertile couples were diagnosed with endometriosis (Mol et al., 1998). In light of these facts, we cannot guarantee that the women in our control group population were free of endometriosis. Thirdly, given that elevated CA-125 levels are associated with other benign or malignant gynaecological diseases (such as ovarian cancer (Fassbender et al.,

2015)), the use of this marker may have induced a degree of clinical heterogeneity (especially in the control group) and might have impacted our results; we did not exclude patients with a history of pelvic inflammation, hydrosalpinx, benign non-endometriotic cysts, or other gynaecological diseases. This also constitutes a source of selection bias.

Thirdly, there were twice as many patients as controls. Although the small size of the control group could be considered as a limitation, it could also be an advantage in view of the absence of laparoscopy in the controls.

Simplification of our model (with a CA-125 cut-off of 30 U/mL (Hirsch, Duffy, Deguara, et al., 2016; Kafali et al., 2004)) would probably decrease the test's accuracy (Nisenblat, Bossuyt, et al., 2016). However, given the study's selection bias (especially in the control group), we consider that our results are promising. Indeed, a meta-analysis showed that for CA-125 alone, the sensitivity ranged from 20% to 50%, and the specificity was higher than 85% (Mol et al., 1998). A more recent meta-analysis found a sensitivity of 52% and a specificity of 93% for all stages of endometriosis (Hirsch, Duffy, Davis, et al., 2016). Our results are similar to the literature data because we obtained a sensitivity of 52% and a specificity of 88%. Moreover, the decision tree derived in the present study (Figure 2) constitutes an efficient, user-friendly tool for identifying a high-risk population. It is noteworthy that more than 80% of the women with a CA-125 level over 30 U/mL (node 9 of the tree, n=87) suffered from endometriosis. Furthermore, 70% of those with a low CA-125 concentration but a marked agglutination reaction (node 8 of the tree, n=65) suffered from endometriosis (Figure 2). Node 8 is particularly interesting because it corresponds to a women with a high risk of endometriosis even though they had a negative result (i.e. <30 U/mL) in the

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

CA-125 assay. The meta-analysis by Hirsch, Duffy, Davis, et al., (2016) showed that endometriosis cannot be ruled out when CA-125 is <30 U/mL.

Our decision tree was able to distinguish two separate populations with a high risk of endometriosis by applying only two parameters: the serum CA-125 level (with the usual cut-off of 30 U/mL) and the sperm agglutination level. Women in either of these subgroups should probably consult an endometriosis specialist. However, our tree was derived from data recorded at a single IVF unit, and its value needs to be confirmed in a multicenter study.

A third parameter (age) was used in the present study but did not appear to be relevant for endometriosis screening. Indeed, the age variable only allowed the subdivision of node 5 (i.e. $CA-125 \leq 30$ U/mL and moderate agglutination [“+”: less than 4 agglutinates per field]) into nodes 6 and 7 (Figure 2). In our regression tree, 30% of the women over the age of 35 years had endometriosis (node 6), whereas more than 50% of women under the age of 35 suffered from endometriosis (node 7). In view of the above observations and the fact that only 30 women were clustered in node 6 and 7, we are therefore not in a position to explain the putative involvement of age in the agglutination reaction.

Our results demonstrated the correlation between sperm agglutination, the serum CA-125 level, and endometriosis. This simple test enables endometriosis screening for high-risk patients in specific populations of women undergoing an IVF procedure. Monitoring sperm agglutination during the IVF procedure and assaying for CA-125 may be of value in patients with IVF failure. A positive test should prompt the patient to consult an endometriosis specialist and perhaps undergo laparoscopy to confirm the presence or absence of endometriosis.

Further research is needed before our strategy can be used in routine clinical practice. This combined analysis can easily be performed in any assisted reproductive technology laboratory, and appears to be a simple, inexpensive way of providing better care and screening for endometriosis in patients undergoing an IVF procedure - especially those with IVF failure and/or idiopathic infertility. The present proof-of-concept study constitutes a first step towards a confirmatory multicentre study with a high-quality control group. It may be ultimately possible to improve the decision tree as a function of to the disease stage or the type of endometriotic lesion.

Acknowledgements

The study was funded by the Maternités et Médecine de la Reproduction Poissy charity (Poissy St Germain, France).

Disclosure statement

The authors declare no competing financial interest or benefit that has arisen from direct applications of this research.

References

- Ballard, K., Lowton, K., & Wright, J. (2006). What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. *Fertility and Sterility*, 86(5), 1296-1301. <https://doi.org/10.1016/j.fertnstert.2006.04.054>
- Ballard, K. D., Seaman, H. E., de Vries, C. S., & Wright, J. T. (2008). Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-control study--Part 1. *BJOG*, 115(11), 1382-1391. <https://doi.org/10.1111/j.1471-0528.2008.01878.x>

- 391 Bedaiwy, M. A., & Falcone, T. (2004). Laboratory testing for endometriosis. *Clinica*
392 *Chimica Acta*, 340(1-2), 41-56. <https://doi.org/10.1016/j.cccn.2003.10.021>
- 393 Blanc, M., & von Theobald, P. (2017). Fertility after surgery for deep infiltrating
394 endometriosis. *Journal of Gynecology Obstetrics and Human Reproduction*,
395 46(2), 143-146. <https://doi.org/10.1016/j.jogoh.2016.12.005>
- 396 Breiman, L., Friedman, J., Stone, C. J., & Olshen, R. A. (1984). *Classification and*
397 *regression trees.*: CRC press.
- 398 Bulletti, C., Coccia, M. E., Battistoni, S., & Borini, A. (2010). Endometriosis and
399 infertility. *Journal of Assisted Reproduction and Genetics*, 27(8), 441-447.
400 <https://doi.org/10.1007/s10815-010-9436-1>
- 401 Coddington, C. C., Oehninger, S., Cunningham, D. S., Hansen, K., Sueldo, C. E., &
402 Hodgen, G. D. (1992). Peritoneal fluid from patients with endometriosis
403 decreases sperm binding to the zona pellucida in the hemizona assay: a
404 preliminary report. *Fertility and Sterility*, 57(4), 783-786.
405 [https://doi.org/10.1016/s0015-0282\(16\)54959-0](https://doi.org/10.1016/s0015-0282(16)54959-0)
- 406 Fassbender, A., Burney, R. O., O, D. F., D'Hooghe, T., & Giudice, L. (2015). Update on
407 Biomarkers for the Detection of Endometriosis. *BioMed Research International*,
408 2015, 130854. <https://doi.org/10.1155/2015/130854>
- 409 Giudice, L. C., & Kao, L. C. (2004). Endometriosis. *Lancet*, 364(9447), 1789-1799.
410 [https://doi.org/10.1016/S0140-6736\(04\)17403-5](https://doi.org/10.1016/S0140-6736(04)17403-5)
- 411 Guerriero, S., Saba, L., Pascual, M. A., Ajossa, S., Rodriguez, I., Mais, V., & Alcazar,
412 J. L. (2018). Transvaginal ultrasound vs magnetic resonance imaging for
413 diagnosing deep infiltrating endometriosis: systematic review and meta-analysis.
414 *Ultrasound in Obstetrics & Gynecology*, 51(5), 586-595.
415 <https://doi.org/10.1002/uog.18961>

- 416 Gupta, D., & Lis, C. G. (2009). Role of CA125 in predicting ovarian cancer survival - a
 417 review of the epidemiological literature. *Journal of Ovarian Research*, 2, 13.
 418 <https://doi.org/10.1186/1757-2215-2-13>
- 419 Hirsch, M., Duffy, J., Davis, C.J., Nieves Plana, M., & Khan, K.S.; International
 420 Collaboration to Harmonise Outcomes and Measures for Endometriosis. (2016).
 421 Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic
 422 review and meta-analysis. *BJOG*, 123(11), 1761-1768.
 423 <https://doi.org/10.1111/1471-0528.14055>
- 424 Hirsch, M., Duffy, J. M., Deguara, C. S., Davis, C. J., & Khan, K. S. (2016). Diagnostic
 425 accuracy of Cancer Antigen 125 (CA125) for endometriosis in symptomatic
 426 women: A multi-center study. *European Journal of Obstetrics, Gynecology, and*
 427 *Reproductive Biology*, 210, 102-107.
 428 <https://doi.org/10.1016/j.ejogrb.2016.12.002>
- 429 Hothorn, T., & Zeileis, A. (2015). partykit: A Modular Toolkit for Recursive
 430 Partytioning in R. *Journal of Machine Learning Research*, 16, 3905-3909.
 431 Retrieved from <http://jmlr.org/papers/v16/hothorn15a.html>
- 432
- 433 Hudelist, G., Fritzer, N., Thomas, A., Niehues, C., Oppelt, P., Haas, D., Tammaa, A., &
 434 Salzer, H. (2012). Diagnostic delay for endometriosis in Austria and Germany:
 435 causes and possible consequences. *Human Reproduction*, 27(12), 3412-3416.
 436 <https://doi.org/10.1093/humrep/des316>
- 437 Kafali, H., Artuc, H., & Demir, N. (2004). Use of CA125 fluctuation during the
 438 menstrual cycle as a tool in the clinical diagnosis of endometriosis; a
 439 preliminary report. *European Journal of Obstetrics, Gynecology, and*

- 440 *Reproductive Biology*, 116(1), 85-88.
- 441 <https://doi.org/10.1016/j.ejogrb.2004.02.039>
- 442 Kuhn, M. (2016). caret: Classification and Regression Training. Retrieved from
- 443 <https://cran.r-project.org/package=caret>
- 444 Liaw, A., & Wiener, M. (2002). Classification and Regression by random Forest. *R*
- 445 *News*, 2(3), 18-22. Retrieved from <http://cran.r-project.org/doc/Rnews/>
- 446 Mansour, G., Aziz, N., Sharma, R., Falcone, T., Goldberg, J., & Agarwal, A. (2009).
- 447 The impact of peritoneal fluid from healthy women and from women with
- 448 endometriosis on sperm DNA and its relationship to the sperm deformity index.
- 449 *Fertility and Sterility*, 92(1), 61-67.
- 450 <https://doi.org/10.1016/j.fertnstert.2008.05.048>
- 451 May, K. E., Conduit-Hulbert, S. A., Villar, J., Kirtley, S., Kennedy, S. H., & Becker, C.
- 452 M. (2010). Peripheral biomarkers of endometriosis: a systematic review. *Human*
- 453 *Reproduction Update*, 16(6), 651-674. <https://doi.org/10.1093/humupd/dmq009>
- 454 Mol, B. W., Bayram, N., Lijmer, J. G., Wiegerinck, M. A., Bongers, M. Y., van der
- 455 Veen, F., & Bossuyt, P. M. (1998). The performance of CA-125 measurement in
- 456 the detection of endometriosis: a meta-analysis. *Fertility and Sterility*, 70(6),
- 457 1101-1108. <https://doi.org/>
- 458 Nisenblat, V., Bossuyt, P. M., Shaikh, R., Farquhar, C., Jordan, V., Scheffers, C. S.,
- 459 Mol, B.W., Johnson, N., & Hull, M. L. (2016). Blood biomarkers for the non-
- 460 invasive diagnosis of endometriosis. *Cochrane Database of Systematic Reviews*,
- 461 5, CD012179. <https://doi.org/10.1002/14651858.CD012179>
- 462 Nisenblat, V., Prentice, L., Bossuyt, P. M., Farquhar, C., Hull, M. L., & Johnson, N.
- 463 (2016). Combination of the non-invasive tests for the diagnosis of

- 464 endometriosis. *Cochrane Database of Systematic Reviews*, 7, CD012281.
 465 <https://doi.org/10.1002/14651858.CD012281>
- 466 Practice Committee of the American Society for Reproductive Medicine. (2015).
 467 Diagnostic evaluation of the infertile female: a committee opinion. *Fertility and*
 468 *Sterility*, 103(6), e44-50. <https://doi.org/10.1016/j.fertnstert.2015.03.019>
- 469 Ram, K., Wickham, H., Richards, C., & Baggett, A. (2015). wesanderson: A Wes
 470 Anderson Palette Generator. Retrieved from [https://cran.r-](https://cran.r-project.org/package=wesanderson)
 471 [project.org/package=wesanderson](https://cran.r-project.org/package=wesanderson)
- 472 Reeve L, Lashen H, Pacey A. A., (2005) Endometriosis affects sperm-endosalpingeal
 473 interactions. *Hum Reprod*. 20(2), 448-451. doi:10.1093/humrep/deh606
- 474 Rogers, P. A., Adamson, G. D., Al-Jefout, M., Becker, C. M., D'Hooghe, T. M.,
 475 Dunselman, G. A., Fazleabas, A., Giudice, L.C., Horne, A.W., Hull, M.L.,
 476 Hummelshoj, L., Missmer, S.A., Montgomery, G.W., Stratton, P., Taylor, R.N.,
 477 Rombauts, L., Saunders, P.T., Vincent, K., & Zondervan, K.T.; WES/WERF
 478 Consortium for Research Priorities in Endometriosis. (2017). Research Priorities
 479 for Endometriosis. *Reproductive Sciences*, 24(2), 202-226.
 480 <https://doi.org/10.1177/1933719116654991>
- 481 Roman, H., Chanavaz-Lacheray, I., Ballester, M., Bendifallah, S., Touleimat, S., Tuech,
 482 J. J., Farella, M., & Merlot, B. (2018). High postoperative fertility rate following
 483 surgical management of colorectal endometriosis. *Human Reproduction*, 33(9),
 484 1669-1676. <https://doi.org/10.1093/humrep/dey146>
- 485 Sáez-Espinosa, P., Velasco, I., Lorca, P., Acien, M. I., Romero, A., & Gomez-Torres,
 486 M. J. (2019). Peritoneal fluid from women with endometriosis impairs human
 487 spermatozoa functionality. *Reproductive Biology*, 20(1), 81-87.
 488 <https://doi.org/10.1016/j.repbio.2019.12.001>

- 489 Sing, T., Sander, O., Beerenwinkel, N., & Lengauer, T. (2005). ROCR: visualizing
 490 classifier performance in R. *Bioinformatics*, 21(20), 3940-3941.
 491 <https://doi.org/10.1093/bioinformatics/bti623>
- 492 Somigliana, E., & Garcia-Velasco, J. A. (2015). Treatment of infertility associated with
 493 deep endometriosis: definition of therapeutic balances. *Fertility and Sterility*,
 494 104(4), 764-770. <https://doi.org/10.1016/j.fertnstert.2015.08.003>
- 495 Somigliana, E., Vigano, P., Benaglia, L., Busnelli, A., Berlanda, N., & Vercellini, P.
 496 (2017). Management of Endometriosis in the Infertile Patient. *Seminars in*
 497 *Reproductive Medicine*, 35(1), 31-37. <https://doi.org/10.1055/s-0036-1597125>
- 498 Spaczynski, R. Z., & Duleba, A. J. (2003). Diagnosis of endometriosis. *Seminars in*
 499 *Reproductive Medicine*, 21(2), 193-208. <https://doi.org/10.1055/s-2003-41326>
- 500 Stilley, J. A., Birt, J. A., & Sharpe-Timms, K. L. (2012). Cellular and molecular basis
 501 for endometriosis-associated infertility. *Cell and Tissue Research*, 349(3), 849-
 502 862. <https://doi.org/10.1007/s00441-011-1309-0>
- 503 Tanbo, T., & Fedorcsak, P. (2017). Endometriosis-associated infertility: aspects of
 504 pathophysiological mechanisms and treatment options. *Acta Obstetrica et*
 505 *Gynecologica Scandinavica*, 96(6), 659-667. <https://doi.org/10.1111/aogs.13082>
- 506 Therneau, T., Atkinson, B., & Ripley, B. (2015). rpart: Recursive Partitioning and
 507 Regression Trees. Retrieved from <https://cran.r-project.org/package=rpart>
- 508 Vinatier, D., Orazi, G., Cosson, M., & Dufour, P. (2001). Theories of endometriosis.
 509 *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 96(1),
 510 21-34. [https://doi.org/10.1016/s0301-2115\(00\)00405-x](https://doi.org/10.1016/s0301-2115(00)00405-x)
- 511 Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (Fourth ed.):
 512 Springer.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*: Springer-Verlag
New York.

Yoshida, S., Harada, T., Iwabe, T., Taniguchi, F., Mitsunari, M., Yamauchi, N., Deura,
I., Horie, S., & Terakawa, N. (2004). A combination of interleukin-6 and its
soluble receptor impairs sperm motility: implications in infertility associated
with endometriosis. *Human Reproduction*, 19(8), 1821-1825.
<https://doi.org/10.1093/humrep/deh324>

Tables captions

Table 1. A qualitative description of the study population.
*The results of the chi-squared test in a bivariate analysis of each of the two dilutions
showed that the CA-125 level and the agglutination level were significantly associated
with the origin of the IVF sample (i.e. endometriosis or not).*

Table 2. The training and test sets.

Table 3. A multivariate model (obtained by logistic regression) for discriminating
between the endometriosis group and the control group.
*All the variables considered here were qualitative. The Akaike information criterion for
the model was 342.33.*

Figures captions

Figure 1. Study design.
*Qualitative microscopic classification of spermatozoon agglutination reactions
(according to the World Health Organization's Laboratory Manual for the Examination*

and Processing of Human Semen, fifth edition), namely: “motile spermatozoa sticking to each other”: (a) no agglutination (-), (b) a low proportion of agglutinated sperm (+), (c) a high proportion of agglutinated sperm (++). The workflow is shown in panel (d).

Figure 2. A Classification and Regression Tree (decision tree).

We developed a decision tree that partitioned the patients into groups that were as homogenous as possible, based on the variable to be predicted (i.e. endometriosis or control). Thus, five groups of patients with different likelihoods of having endometriosis (ranging from less than 30% to over 80%) were obtained by considering the agglutination level, the CA-125 level, and the age class. These groups are represented by the tree’s end nodes. The variable referred to as Agglutination 1:4 corresponds to the proportion of sperm agglutination obtained with a 1:4 dilution of the serum, classified as: “-”: a low proportion of agglutinated sperm; “+”: less than 4 agglutinates per field); “+ +”: a high proportion of agglutinated sperm, 4 or more agglutinates per field. The cut-off for the serum CA-125 level was 30 U/mL (i.e. ≤ 30 U/mL vs. > 30 U/mL). The cut-off for age was 35 (i.e. < 35 vs. ≥ 35). After the decision tree had been applied to the test set, we determined the model’s sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

555

Supporting information (see Supplementary files)

557

S1 Fig. Data distribution.

A) Boxplots for each group, showing the data distribution for age and CA-125 concentration (logarithmic scale); B) Data distribution for quantitative variables (age and CA-125). The groups are shown in different colours. The two groups’ baseline characteristics were compared using the Mann-Whitney test. There were statistically

1
2
3 563 *significant associations between the CA-125 concentration and endometriosis ($p =$*
4
5 564 *1.021×10^{-9}) and between age and endometriosis ($p = 0.03595$).*
6
7 565
8
9
10 566 *S2. Fig. A) Variable importance in the model, tested in a random forest analysis.*
11
12 567 *Agglutination 1:4 was the most important variable in this model, with regard to both*
13
14 568 *accuracy and the Gini index. B) The random forest plot for 200 trees, mtry= 3, and an*
15
16 569 *estimated OOB error rate of 23.72%.*
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

Table 1. A qualitative description of the study population.

The results of the chi-squared test in a bivariate analysis of each of the two dilutions showed that the CA-125 level and the agglutination level were significantly associated with the origin of the IVF sample (endometriosis or not).

Variable	Control group		Endometriosis group		Total		χ^2	p-value
	n	%	n	%	n	%		
Age class								
≤ 35 years	50	48.08	124	59.62	174	55.77	3.289	0.07
> 35 years	54	51.92	84	40.38	138	44.23		
CA-125 level								
≤ 30 U/mL	87	83.65	108	51.92	195	62.5	28.446	<0.0001***
> 30 U/mL	17	16.35	100	48.08	117	37.5		
Agglutination with a 1:2 dilution								
-	21	20.19	18	8.65	39	12.5	16.302	<0.0005**
+	28	26.92	34	16.35	62	19.87		

++	55	52.88	156	75	211	67.63		
Agglutination with a 1:4 dilution								
-	44	42.31	30	14.42	74	23.72	36.863	<0.0001***
+	25	24.04	40	19.23	65	20.83		
++	35	33.65	138	66.35	173	55.45		

Table 2. The training and test sets.

Variable	Training set						Test set					
	Control		Endometriosis		Total		Control		Endometriosis		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
Age range												
≤ 35 years	36	49.32	86	58.9	122	55.71	14	45.16	38	61.29	52	55.91
> 35 years	37	50.68	60	41.1	97	44.29	17	54.84	24	38.71	41	44.09
CA-125 level												
≤ 30 U/mL	61	83.56	71	48.63	132	60.27	26	83.87	37	59.68	63	67.74
> 30 U/mL	12	16.44	75	51.37	87	39.73	5	16.13	25	40.32	30	32.26

Agglutination with a 1:2 dilution												
-	14	19.18	12	8.22	26	11.87	7	22.58	6	9.68	13	13.98
+	21	28.77	26	17.81	47	21.46	7	22.58	8	12.9	15	16.13
++	38	52.05	108	73.97	146	66.67	17	54.84	48	77.42	65	69.89
Agglutination with a 1:4 dilution												
-	31	42.47	23	15.75	54	24.66	13	41.94	7	11.29	20	21.51
+	19	26.03	28	19.18	47	21.46	6	19.35	12	19.35	18	19.35
++	23	31.51	95	65.07	118	53.88	12	38.71	43	69.35	55	59.14

571

572

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

Table 3. Multivariate model (obtain by logistic regression) for discriminating between the endometriosis group and the control group.

All the variables considered here were qualitative.

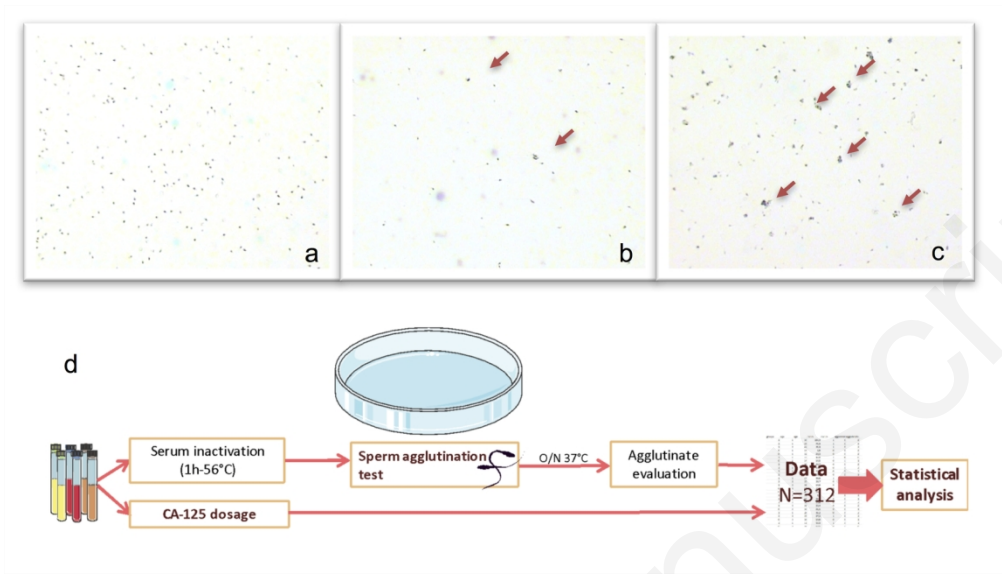
The Akaike information criterion for the model was 342.33.

	Odds ratio	95%CI	p value
(Intercept)	0.5513037	0.3091266 – 0.9630029	>0.01
CA-125 > 30 U/mL	4.3015392	2.3748690 – 8.1532708	<0.0001
Agglutination1:4 +	2.2483028	1.0959224 – 4.6887153	>0.01
Agglutination1:4 ++	5.3711769	2.9005988 – 10.1558972	<0.0001
Age > 35	0.6644742	0.3906826 – 1.1264008	>0.1

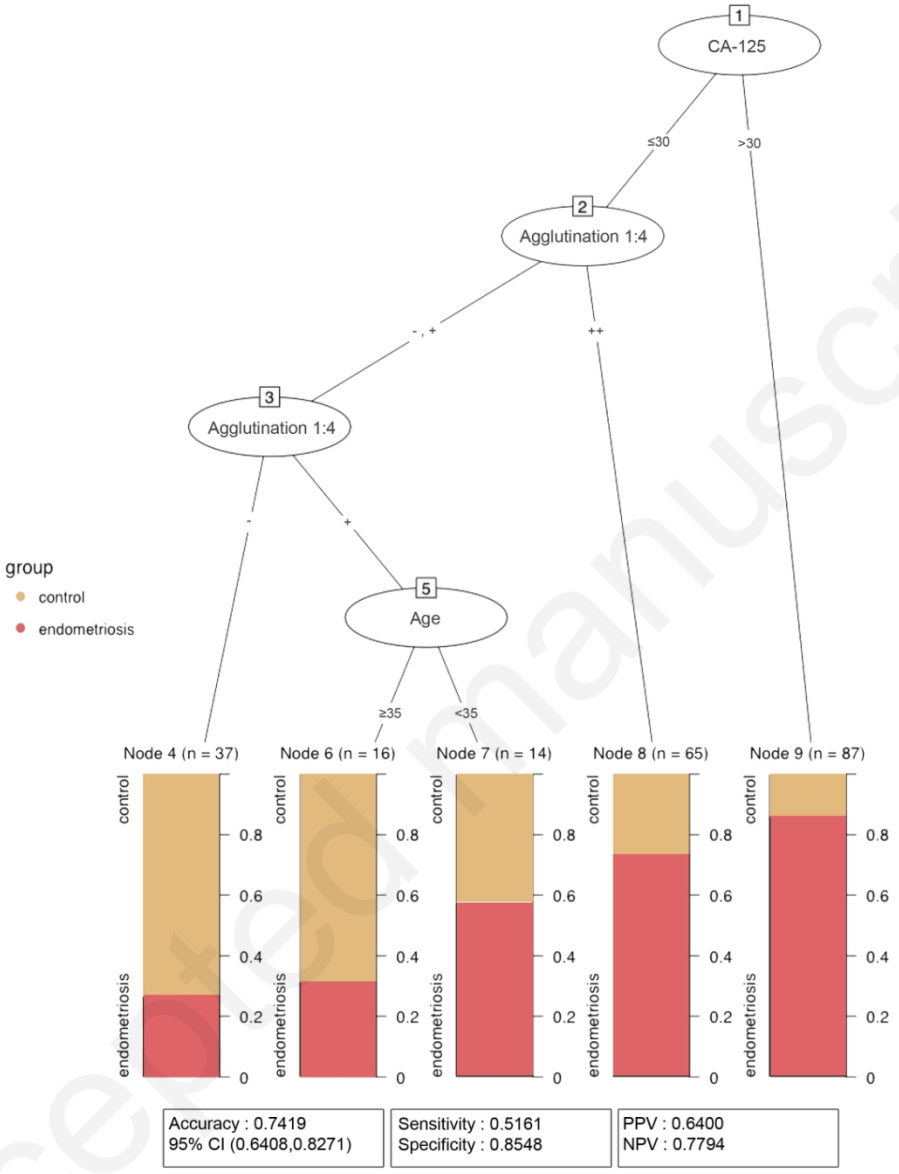
*

*

573

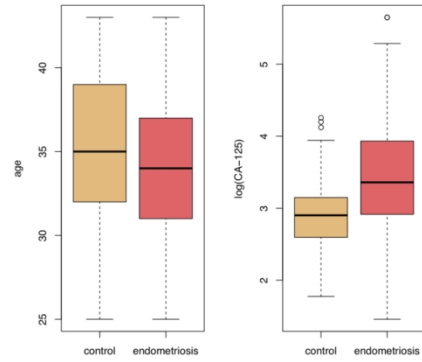


149x85mm (300 x 300 DPI)

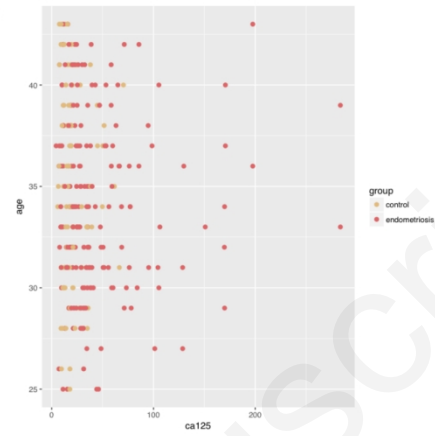


99x123mm (300 x 300 DPI)

A



B



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

