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Predictors of Patients Remaining Anovulatory during Clomiphene Citrate Induction of Ovulation in Normogonadotropic Oligoamenorrheic Infertility*

BABEK IMANI, MARINUS J. C. EIJKEMANS, EGBERT R. TE VELDE, J. DIK F. HABBEMA, AND BART C. J. M. FAUSER

Division of Reproductive Medicine, Department of Obstetrics and Gynecology (B.I., B.C.J.M.F.), and Department of Public Health (M.J.C.E., J.D.F.H.), Center for Clinical Decision Sciences, University Hospital and Erasmus University, Rotterdam; and the Department of Obstetrics and Gynecology, University Hospital Utrecht (E.R.t.V.), Utrecht, The Netherlands

ABSTRACT

The diagnostic criteria used to identify patients suffering from polycystic ovary syndrome remain controversial. The present prospective longitudinal follow-up study was designed to identify whether certain criteria assessed during standardized initial screening could predict the response to ovulation induction with clomiphene citrate (CC) in 201 patients presenting with oligomenorrhea or amenorrhea and infertility. Serum FSH levels were within the normal range (1–10 IU/L), and all patients underwent spontaneous or progestin-induced withdrawal bleeding. Initial CC doses were 50 mg daily for 5 days starting on cycle day 3. In the case of an absent response, doses were increased to 100 and 150 mg daily in subsequent cycles. First ovulation with CC was used as the end point. After a complete follow-up (in the case of a nonresponse, at least 3 treatment cycles with daily CC doses up to 150 mg), 156 patients (78%) ovulated. The free androgen index (FAI = testosterone/sex hormone-binding globulin ratio), body mass index (BMI), cycle history (oligomenorrhea *vs.* amenorrhea), serum androgen (testosterone and/or androstenedione) levels, and mean ovarian volume assessed by transvaginal sonography were all significantly different ($P < 0.01$) in responders from those in nonresponders. FAI was chosen to be the best predictor in univariate analysis. The area under the receiver operating characteristics curve in a multivariate prediction model including FAI, BMI, cycle history, and mean ovarian volume was 0.82.

Patients whose ovaries are less likely to respond to stimulation by FSH due to CC treatment can be predicted on the basis of initial screening characteristics, such as FAI, BMI, cycle history (oligomenorrhea or amenorrhea), and mean ovarian volume. These observations may add to ongoing discussion regarding etiological factors involved in ovarian dysfunction in these patients and classification of normogonadotropic anovulatory infertile women. (*J Clin Endocrinol Metab* **83:** 2361–2365, 1998)

CHRONIC anovulation is a frequent cause of infertility, and approximately 80% of these patients present with serum FSH and estradiol levels within the normal range (WHO group 2) (1). The antiestrogen clomiphene citrate (CC) is considered to be a successful treatment strategy in these patients. It has been documented that approximately 70–80% of these women will become ovulatory (2–7), whereas 40–50% of ovulatory women will conceive (3, 5).

Polycystic ovary syndrome (PCOS), usually referred to as chronic hyperandrogenic anovulation, represents a distinct proportion of WHO group 2 anovulatory patients. It is uncertain to what extent PCOS patients are particularly prone to remain resistant to CC medication (8, 9). Discussion continues regarding the validity of criteria used to diagnose PCOS (10) as well as its relevance for clinical practice. We have previously demonstrated that a distinct overlap exists between endocrine and ultrasound features used by various researchers (11). If strict criteria are used for PCOS diagnosis, a large heterogeneous group of normoestrogenic patients will remain unclassified.

Thirty-five years after its first clinical introduction (12), CC still remains the first line treatment strategy in normogonadotropic anovulatory patients. Although rising serum FSH levels due to CC interference with estrogen negative feedback may be held responsible for stimulating follicle growth (13–15), other mechanisms of action have also been proposed (9, 16). A significant proportion of treated women, however, do not respond. The aim of this study was to investigate whether clinical, endocrine, and sonographic characteristics during initial screening of normogonadotropic anovulatory infertile women may predict the ovarian response to CC medication. This approach may help to define conditions that prevent the ovary from responding to stimulation by increased FSH levels and to further classify WHO group 2 anovulatory patients.

Subjects and Methods

Subjects and study design

Approval for this study was obtained from the human subject committee of the Dijkzigt Hospital/Erasmus University. Between February 1993 and September 1996, 201 patients presenting with oligomenorrhea (interval between vaginal bleeding >35 days and <6 months) or amenorrhea (bleeding interval >6 months) and infertility were recruited. Informed consent was obtained from all participants. All subjects were referred directly by their general practitioner to our infertility unit. None

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Address all correspondence and requests for reprints to: Prof. B. C. J. M. Fauser, M.D., Ph.D., Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Dijkzigt Academic Hospital, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. E-mail: fauser@gyna.azr.nl.

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had received previous ovulation induction medication. Additional inclusion criteria were serum FSH levels within normal limits (1–10 IU/L) (1, 17), spontaneous menses or a positive bleeding response to progestagen withdrawal, normal serum PRL and THS levels, body mass index (BMI; weight divided by the square of the patients height) more than 18, and age less than 40 yr.

Clinical, endocrine, and sonographic screening was carried out before initiation of CC therapy. Clinical screening included infertility and cycle history, BMI, previous medication, and/or surgery. Endocrine screening included serum assays of FSH, PRL, TSH, LH, estradiol, androstenedione (AD), testosterone (T), sex hormone-binding globulin (SHBG), cortisol, and dehydroepiandrosterone sulfate. Fasting blood samples were taken randomly between 0800–1000 h before the initiation of therapy. Venous blood samples were centrifuged within 2 h after withdrawal and were stored at −20 C until assayed. Transvaginal sonographic screening included assessment of the ovarian stroma echogenicity (arbitrarily classified from 1–3), ovarian volume (milliliters), and total number of follicles (both ovaries), as described previously (11, 18). Serum LH and FSH levels were measured by immunoradiometric assay (Medgenix, Fleurus, Belgium), and T, AD, SHBG, and dehydroepiandrosterone sulfate were determined using RIA kits (Diagnostic Products Corp., Los Angeles, CA), as described previously (19, 20).

The treatment schedule and assessment of ovarian response were as follows. CC medication was initiated on day 3 after spontaneous or progestagen-induced withdrawal bleeding. The starting dose was 50 mg/day, orally, for 5 subsequent days. In the case of an absent response, daily doses were increased by 50 mg in the next cycle to a maximum dose of 150 mg/day in the following cycle. If ovulation occurred, the dose remained unaltered during subsequent cycles. First ovulation was used as the end point. The duration of follow-up for all patients included in the study was at least three treatment cycles. Ovulation was assessed by midluteal serum progesterone measurement (levels >25 nmol/L indicating ovulation) combined with transvaginal sonographic monitoring of follicle growth until the appearance of a preovulatory follicle (mean diameter, ≥18 mm) and subsequent follicle rupture, or by biphasic basal body temperature charts. Responders were defined as patients who ovulated during CC therapy, independent of the dose administered. The number of treatment cycles and the CC dose in which first ovulation occurred were recorded. Clomiphene-resistant anovulation (CRA) was defined as patients who do not ovulate despite receiving maximum treatment doses of 150 mg/day.

Data analysis

Distribution of characteristics in patient groups is presented as the mean ± SD. We used the Mann-Whitney U test and the Wilcoxon rank sum W test for exploratory comparison of initial parameters between responders and nonresponders. The univariate and multivariate relation with response to CC was assessed using logistic regression analysis. The following parameters were used in the analysis: BMI, free androgen index (FAI; T × 100/SHBG), serum T and/or AD concentrations, serum LH levels, cycle history (oligomenorrhea or amenorrhea), cycle duration in case of oligomenorrhea, mean ovarian volume, and follicle number. Backward stepwise elimination was used for the multivariate logistic analysis of prediction of patients being CRA, and $P \leq 0.10$ was used as a cut-off level for elimination of nonsignificant predictors from the prognostic model. The area under the receiver operating characteristics (ROC) curve (AUC) was used to assess the discriminative ability of the logistic models. The AUC gives the proportion of all pairs of patients (each pair consisting of one patient without and the other patient with a response to CC) in which the model predicts a higher probability of no response for the patient without response. The Statistical Analysis System program (SAS Institute, Cary, NC) was employed for data analysis.

Because selection and estimation were performed for 8 potential predictors on a dataset with only 45 events (CRAs), correction for overfitting was performed (21). The internal validity of the prognostic model was tested by a bootstrapping method in which the selection and estimation process was repeated 200 times. Each of these repetitions consisted of creating a new dataset (bootstrap sample) by drawing cases with replacement from the original data. The backward stepwise elimination process was performed on this dataset, yielding a set of selected predictors and parameter estimates (21, 22). Resulting model estimates of each bootstrap sample were evaluated on the original data, and a shrinkage factor was estimated to correct for statistical overoptimism. The Hosmer-Lemeshow goodness of fit test (23) has been used to check for lack of fit of the final model. The logistic coefficients that were corrected by the shrinkage factor have been translated into an easy to use score chart. The scores were calculated by multiplying the shrunk coefficients by 10 and rounding them off to the nearest whole number.

Results

The number of patients who did or did not ovulate after CC medication in increasing doses of 50, 100, and 150 mg daily are depicted in Fig. 1. Forty-five patients (22.5% of the overall study group) remaining anovulatory were considered having CRA. A total of 432 cycles were analyzed.

From the total study group of 201 women, 91 (46%) were considered obese (BMI >26), 101 patients (50%) presented with an elevated FAI (>4.5), 85 patients (42%) presented with hyperandrogenemia (T ≥3.2 nmol/L and/or AD ≥16.3 nmol/L) (11, 19), and in 125 patients (66%) polycystic ovaries (mean ovarian volume ≥10.8 mL and/or mean follicle number per ovary ≥10) (11, 18) were diagnosed. Finally, 105 patients (54%) presented with elevated LH (≥7.0 IU/L) serum levels (11, 19).

In Table 1, clinical, endocrine, and ultrasound characteristics are presented for the overall study group and separately for patients who did or did not ovulate after CC medication. Forty-four percent of patients presenting with amenorrhea (17 of 39) were considered to have CRA, whereas only 17% (28 of 162) of patients with oligomenorrhea showed no response. Statistical significance in univariate analysis with logistic regression analyses and ROC AUC of the initial parameters are depicted in Table 2. The AUCs for FAI and BMI were the highest (0.76 and 0.70, respectively). The ROC curve with the best performance (FAI) and that with the poorest performance (serum LH) are shown in Fig. 2.

Of the 201 patients, 187 had complete data on the variables used in the multivariate analysis. Using the backward elimination procedure, 4 variables were selected in the final model: 1) FAI, 2) BMI, 3) cycle history (oligomenorrhea or amenorrhea), and 4) mean ovarian volume. By using the

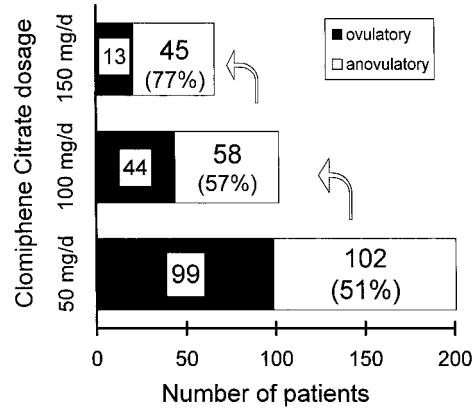

FIG. 1. Distribution of normogonadotropic oligomenorrheic or amenorrheic infertile women who do or do not ovulate after CC induction of ovulation in incremental daily doses of 50, 100, or 150 mg for 5 subsequent days. A total of 45 women (22.5% of the overall study group) remain anovulatory.

TABLE 1. Clinical, endocrine, and ultrasound characteristics (mean ± SD) during initial screening of 201 normogonadotropic oligomenorrheic or amenorrheic infertile women, and separated for patients who do (responders) or do not ovulate (CRA) after CC induction of ovulation

Screening parameters	Overall group (n = 201)	CC responder (n = 156; 77.5%)	CRA (n = 45; 22.5%)	*P* value[a]
Clinical				
Age (yr)	28 ± 4.4	28 ± 4.5	27.5 ± 4.5	NS
Primary infertility (n)	145 (72%)	110 (71%)	35 (78%)	NS
Amenorrhea (n)	39 (19%)	22 (14%)	17 (38%)	0.0004
Bleeding interval (days, in case of oligomenorrhea)	79 ± 62	70 ± 56	113 ± 72	<0.0001
BMI (kg/m^2)	26.6 ± 6.2	25.5 ± 5.8	30.0 ± 6.6	0.0001
Endocrine				
T (nmol/L)	2.3 ± 0.9	2.1 ± 0.9	2.7 ± 1.0	0.001
AD (nmol/L)	16.5 ± 7.8	15.3 ± 6.5	20.5 ± 10.2	0.001
SHBG (nmol/L)	53 ± 31.7	57 ± 32.5	38.3 ± 23.8	<0.0001
FAI (T × 100/SHBG)	5.9 ± 4.3	4.9 ± 3.4	9.3 ± 5.3	<0.0001
LH (IU/L)	7.8 ± 4.3	7.8 ± 4.3	8.2 ± 4.6	NS
FSH (IU/L)	4.4 ± 1.4	4.5 ± 1.4	4.2 ± 1.4	NS
E_2 (pmol/L)	282 ± 233	296 ± 195	234 ± 78	NS
DHEAS (μmol/L)	7.9 ± 3.8	7.9 ± 3.7	7.9 ± 4	NS
TVS				
Total stroma score[b]	3.0 ± 1.0	2.8 ± 1.3	3.3 ± 1.1	0.006
Mean ovarian vol (mL)	10.0 ± 4.4	9.2 ± 5.7	12.2 ± 5.8	0.0007
Mean follicle no.	11.5 ± 5	11 ± 6	12 ± 5	NS

[a] Comparison of CC responders *vs.* CRA (by Mann-Whitney U test).
[b] Arbitrarily defined as one to three per ovary (both ovaries added).

TABLE 2. Univariate and multivariate logistic regression analyses with score test and area under the ROC curve (AUC) of initial clinical, endocrine, and sonographic screening parameters in 201 normogonadotropic oligomenorrheic or amenorrheic infertile women for the prediction of patients remaining anovulatory after CC induction of ovulation

Parameters	*P* value	AUC[a]
Univariate analyses		
FAI (T × 100/SHBG)	<0.0001	0.76
BMI (kg/m^2)	<0.0001	0.70
Mean ovarian vol	0.0001	0.67
Hyperandrogenemia (elevated T and/or AD)	0.0007	0.64
Oligomenorrhea or amenorrhea	0.0005	0.62
Mean follicle no.	0.1	0.58
Bleeding interval in case of oligomenorrhea	0.42	0.53
LH (IU/L)	0.5	0.52
Multivariate analysis		
Prediction model for CRA[b]		0.82

[a] Area under the ROC curve.
[b] Combination of four initial screening parameters: FAI, BMI, cycle history (oligomenorrhea or amenorrhea), and mean ovarian volume.

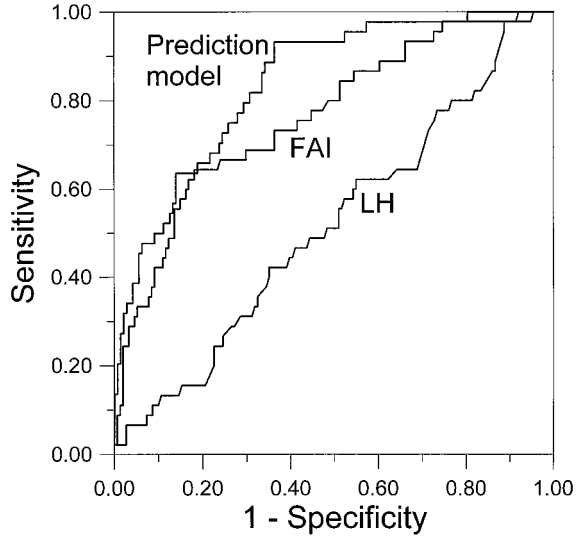

FIG. 2. ROC curve of serum LH concentration, FAI, or the prediction model (FAI, BMI, cycle history, and mean ovarian volume combined) for predicting CRA in a total group of 201 normogonadotropic oligomenorrheic or amenorrheic infertile women.

combined information of these 4 variables, the AUC further improved to 0.82 (Table 2 and Fig. 2). The bootstrap procedure revealed that these 4 predictors were selected in over two thirds of the bootstrap samples, whereas all other candidate variables were selected in less than half of the samples, which illustrates the stability of the final model. The shrinkage factor was estimated from the bootstrap procedure to be 0.82, indicating that when this study is replicated many times, the resulting coefficients of the final multivariate model are, on the average, 18% smaller. This was incorporated in the calculation of the scores. The scores for different parameters are depicted in Table 3, and resulting probability scores for patients remaining anovulatory after CC medication are shown in Fig. 3. The Hosmer-Lemeshow goodness of fit test showed no lack of fit of the final model to the data ($P = 0.3$).

Discussion

It may be helpful for further classification of normogonadotropic anovulatory infertility and for evaluating pathophysiological factors involved in ovarian abnormalities in these patients to study in a longitudinal fashion whether initial screening parameters may predict success, failure, or complications of induction of ovulation. The present study was designed to investigate as a first step whether ovarian response after CC medication could be predicted. It is established in the literature that approximately 75% of patients will ovulate, and less than 50% of the total population will conceive after CC as first line medication. If patients remaining anovulatory despite CC therapy could be identified beforehand, ineffective and

TABLE 3. Score charts of four initial screening parameters of the final model for prediction of patients remaining anovulatory after CC induction of ovulation in normogonadotropic oligomenorrheic or amenorrheic infertile women (total scores, 0–53)

Initial screening parameters	Score[a]
FAI (T × 100/SHBG)	
<2	0
2–3	1
3–4	2
4–5	3
5–6	5
6–8	6
8–11	10
>11	14
BMI (kg/m^2)	
<20	0
20–21.5	2
21.5–23	3
23–25	4
25–27	6
27–31	8
31–35	12
>35	15
Mean ovarian vol (mL)	
<6	0
6–7	2
7–8	2
8–9	3
9–11	5
11–13	6
13–16	9
>16	11
Cycle history	
Oligomenorrhea	0
Amenorrhea	13
Total score	—

[a] Encircle the scores related to each category of the screening parameters and add them together. Correspond the total score to the score chart (Fig. 3). As an example, a new amenorrheic patient had the following findings: FAI = 8.7, BMI = 29.4, and mean ovarian volume = 13 ml. Scores are 10 for FAI, 8 for BMI, 9 for mean ovarian volume, and 13 for having amenorrhea. The total score is 40, and the corresponding probability to be CRA is 65%.

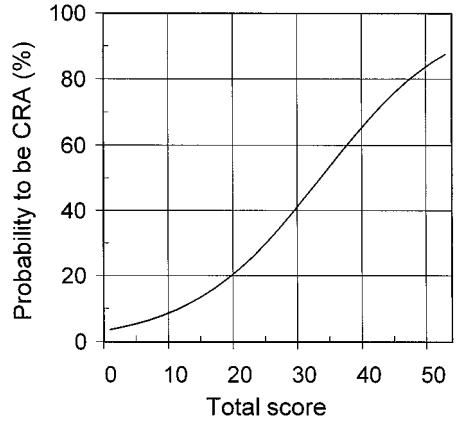

FIG. 3. Score chart and the probability for a given woman to remain anovulatory after CC induction of ovulation.

time-consuming CC treatment could be prevented. This may be helpful, particularly for women of advanced reproductive age. Further studies, however, are required to investigate whether alternative primary treatment options are cost effective. Moreover, etiological factors involved in ovarian dysfunction could be identified in this heterogeneous patient group, as subjects whose ovaries will or will not respond to increased FSH stimulation may be differentiated.

For this first analysis of response to CC medication we decided to focus on ovulation rather than conception. Ovulation is biologically relevant and most closely connected to the desired effects of CC medication. Analysis of conception as the end point requires a comprehensive study of other potential confounders, such as tubal factor, sperm factor, and endometrial function. Four initial screening parameters (FAI, BMI, mean ovarian volume, and cycle history) could be identified, predicting patients remaining anovulatory after CC medication. A combination of these parameters showed good predictive power, with a ROC AUC of 0.82. Several studies have been published recently regarding the use of a similar multivariate model for predicting chances for conception in infertile patients with regular cycles (24–26). Various researchers have investigated the predictive value of clinical and endocrine screening parameters for the response to CC. Only a positive correlation between body weight and the dose of CC required to induce ovulation has been established (8, 27). A recent study has indicated that increased BMI is the only initial parameter that is significantly different between responders and nonresponders (28). To our knowledge this is the first time a multivariate prediction model has been applied in the treatment of anovulatory infertility. We could demonstrate that patients suffering from amenorrhea, obesity, increased ovarian volume, and elevated androgen levels (a complex of signs, symptoms, and ultrasound and endocrine findings frequently referred to as PCOS) are most likely to remain anovulatory after CC induction of ovulation. A model can be used to predict chances for an individual patient to remain anovulatory by calculating a total score on the basis of these initial screening characteristics. Further studies should validate the prediction model in a new group of patients. The present study also suggests that LH concentrations do not predict ovarian response after CC medication in accordance with recent observations by others (28). These data oppose the concept that elevated LH is implemented in ovarian dysfunction in these patients (29). However, the assessment of LH levels in anovulatory patients is problematic due to effects of timing, the immunoassays used, and the pulsatile nature of LH release (30).

Together, these observations suggest that obese hyperandrogenic women are less likely to respond to increased stimulation by FSH, suggesting that these factors are instrumental in follicle maturation arrest (31) and an increased FSH threshold (6). The correlation between BMI and ovarian response after CC treatment suggests that much emphasis should be focused on weight reduction. However, it should be realized that scientific proof for this approach is lacking, and that weight reduction may not necessarily result in normal response. Previous work from our group demonstrated indeed that long term androgen medication in female to male transsexuals induces polycystic ovary morphology, characterized by increased ovarian size, augmented follicle number, and stroma hyperplasia (32). Assessment of steroid levels in follicle fluid obtained from polycystic ovaries suggested that disturbed dominant follicle selection in hyperandrogenic patients may result from disrupted enhancement of

FSH-induced aromatase activity (33). This could be due to intraovarian dysregulation of FSH action, which precludes a normal response (*i.e.* follicle growth and ovulation) after incremental FSH levels elicited by CC medication. Factors involved may include locally produced growth factors or insulin resistance (34, 35). Alternative explanations for a nonresponse after CC may include 1) an abnormal hypothalamic/pituitary response to steroid feedback resulting in an insufficient rise in FSH after CC, 2) individual differences in the FSH isohormone profile resulting in discrepancies in bioactive FSH concentrations despite similar immunoreactive FSH levels, 3) additional, as yet unidentified, mechanisms responsible for at least part of CC actions. Recently reported CC-induced changes in the insulin-like growth factor system (16) may be relevant in this regard. It may be speculated that improved insight into any of these factors (intraovarian dysregulation (36), hypothalamic/pituitary dysfunction (37), FSH heterogeneity (38), or the insulin-like growth factor system (34)) may eventually result in additional predictors of the CC response. Further studies focusing on insulin resistance may also be of interest, as recent reports suggest that ovarian dysfunction may improve after the use of insulin-sensitizing agents (39). Preliminary observations (Imani, B. and Fauser, B. C. J. M., unpublished observations) suggest that fasting insulin levels are increased in CRA patients. It requires further study to clarify whether insulin resistance is a determining factor in an abnormal response to FSH independent from androgen concentrations, as the majority of studies propose that insulin resistance is associated with PCOS through increased thecal cell androgen production (35).

In conclusion, this study demonstrates that it is possible to predict patients remaining anovulatory during CC induction of ovulation using criteria that are directly associated with PCOS, predominantly obesity and hyperandrogenemia. Further studies should establish whether the occurrence of pregnancies after CC medication can also be predicted and whether similar factors are involved. The identification of initial characteristics that predict the ovarian response to ovulation induction therapy may help to further classify the heterogeneous group of normogonadotropic anovulatory infertile women. The present study suggests that hyperandrogenemia and obesity are crucial in inducing ovarian abnormalities that are less likely to respond to increased stimulation by FSH.

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