Response to ovulation induction treatments in women with polycystic ovary syndrome as a function of serum anti-Müllerian hormone levels

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Abstract

Purpose To assess whether anti-Müllerian hormone (AMH) can predict response to ovulation induction (OI) with clomiphene citrate (CC), letrozole (LET), or follicle-stimulating hormone (FSH) in women with polycystic ovary syndrome (PCOS) undergoing OI/intrauterine inseminations (IUI).

Methods A total of 738 OI/IUI cycles from 242 patients at an academic center were stratified in three groups by medication: CC ($n = 295$), LET ($n = 180$), and FSH ($n = 263$), in a retrospective fashion. Ovarian response to treatment (RT, development of at least one dominant follicle) was assessed using mixed effects logistic regression models.

Results Overall, RT cycles had lower AMH levels compared to no-RT cycles ($p < 0.001$). This finding persisted when analysis was limited to oral agents but attenuated in FSH cycles. For CC and LET cycles, the predicted probability (PProb) for RT decreased as AMH levels increased (PProb (95%CI): 97% (93–100), 79% (70–88), and 75% (61–89); 85% (78–93), 75% (67–83), and 73% (63–86) for AMH pct.: ≤ 25th, ≥ 50th, and ≥ 75th, for CC and LET, respectively). However, RT was noted in 98.5% of FSH/IUI cycles regardless of AMH. For CC cycles, those with AMH ≥ 75th pct. had lower odds for RT over cycles with AMH < 75th pct. (OR 0.2, 95%CI 0.04–0.8, $p = 0.02$). Similarly, lower odds for RT were observed in LET cycles with AMH ≥ 75th pct. (0.6, 0.3–1.4, $p = 0.25$).

Conclusion In PCOS, increasing serum AMH levels are associated with lower probability of RT to oral agents. Our findings constitute a valuable tool for the clinician when counseling PCOS patients and designing a personalized ovulation induction treatment strategy.

Keywords PCOS · Ovulation induction · Treatment response · AMH

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Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent cause of oligo-anovulation, commonly seen among reproductive-aged women [1], and couples with infertility [2, 3]. Among the latter group, ovulation induction (OI) is often the first line of treatment and a few OI options are available, including clomiphene citrate (CC), letrozole (LET), and/or gonadotropins [4].

Since its introduction as an OI agent in 1961, CC became a popular treatment mainly because of its affordable cost, excellent safety profile, and ease of use [5, 6]. However, its overall low chance of success (measured both as ovulatory response and live birth rates) and its associated risk of multiple pregnancy (3–8%) and cyst formation [7], as well as its anti-estrogenic effects on the endometrium [4, 8–10], necessitated

alternative treatment options. In recent years, aromatase inhibitors emerged as an alternative, and letrozole (LET) was initially utilized in cases of CC resistance. LET became a preferred treatment for OI in PCOS patients due to a growing body of evidence suggesting that stimulation with LET, as opposed to CC, is associated with higher rates of ovulation, pregnancy, and cumulative live birth, and a lower risk of multiple pregnancy [11–15]. Alternatively, gonadotropins may successfully stimulate follicular growth in PCOS patients, albeit their use is associated with increased cost and more frequent monitoring. Among patients with idiopathic infertility, on the other hand, gonadotropins are associated with higher rates of live birth at the expense of a higher rate of multiples when compared to CC and LET [16]. At the present time, establishing personalized treatment plans for ovulation induction remains challenging mostly because specific patient characteristics that best predict response to treatment have not been elucidated [17–22].

In the recent years, anti-Müllerian hormone (AMH), a dimeric glycoprotein which is usually elevated in women with PCOS [23, 24], emerged as a candidate marker with possible value in predicting OI outcomes among PCOS patients [25–27]. Among women undergoing assisted reproductive technology treatment, it correlates positively with ovarian response, and at substantially higher levels it also correlates with the risk for ovarian hyperstimulation syndrome [28, 29]. A negative association between high AMH levels and response to OI with either CC or LET has also been speculated [25–27, 30]. However, given the absence of convincing evidence regarding the association between AMH and response to OI agents, serum AMH levels are not taken into consideration in the selection of OI treatment protocols.

The objective of this study was to evaluate the association, if any, between pre-treatment serum AMH levels and follicular response to different OI regimens among women with PCOS. We hypothesized that in cycles characterized by higher pre-treatment serum AMH levels, response to treatment might differ between OI regimens (CC, LET, and FSH).

Materials and methods

Study design

The study was conducted at Massachusetts General Hospital (MGH) and was approved by the Partners Healthcare Institutional Review Board. Data from 13,588 OI/intrauterine insemination cycles, occurring between 4/2003 and 8/2019 at the MGH Fertility Center, were reviewed and patients with the diagnosis of PCOS and available pre-treatment serum levels of AMH were included in the current analysis. The diagnosis of PCOS was based on the Rotterdam criteria [31]. Cycles from women without documented pre-treatment serum AMH levels were excluded from the analysis (most performed prior to 2013).

Ovulation induction protocols

Starting dose for most CC and LET cycles was 50 and 2.5 mg, respectively, with instructions to take for 5 days starting on cycle days 3–5 (post spontaneous menstruation or progesterone withdrawal bleed). Response was monitored via transvaginal ultrasonography with baseline ultrasound performed on cycle day 3 and the first follicular phase ultrasound performed on cycle days 10–12. Monitoring frequency after that was individualized until the dominant follicle reached pre-ovulatory measurements. Similarly, in cycles utilizing gonadotropins, injections were started on the 3rd cycle day. Initial dose was determined by the patient's fertility physician, who took into consideration the patient's age, BMI, ovarian reserve biomarkers, and prior response, when available. Follicular development was then monitored by transvaginal ultrasonography and serum estradiol (E_2) measurements, performed at regular intervals. The dose of gonadotropins was then adjusted accordingly. Ovulation was triggered with 250 µg of recombinant human chorionic gonadotropin (hCG) (Ovidrel; Serono Laboratories, Norwell, MA), when at least one dominant follicle ($\geq$ 16mm) was identified. Cycles with development of at least one dominant follicle consisted the study's "*response to treatment*" group, whereas cycles with no follicular response were canceled and consisted the study's "*no response to treatment*" group. In the latter group, the treatment dose was usually increased in subsequent cycles in a "step-up" fashion (100 or 150 mg CC, 5 or 7.5 mg letrozole), unless there was either a reason to believe that the patient will benefit from another OI agent or at the patient's request. In a smaller portion of cycles that failed to respond to oral agents, gonadotropins were used in a subsequent cycle. The selection of gonadotropins was triggered by various factors (patient's preference, restricted insurance coverage, side effects from the original OI, or negative impact on the endometrium, etc.).

As previously reported, prior to treatment initiation, all couples underwent a standard infertility evaluation [32], identifying at least one open fallopian tube and post-processing total motile sperm counts $\geq$ 1 million. All recorded values, related to patient's characteristics, were abstracted from the patients' electronic record, and measured within the year preceding the initiation of each treatment cycle. Prior to January 2018, AMH levels were measured at Mayo Clinic Department of Laboratory Medicine and Pathology (Rochester, MN) by the Ansh Labs ultrasensitive AMH/Müllerian-inhibiting substance enzyme-linked immunosorbent assay (ELISA) (package insert:

Ansh Labs Ultra-Sensitive AMH; document AL-105; revision no.04), and later at the Brigham and Women's Hospital Laboratory of Pathology of Boston, using the Elecsys AMH immunoassay by Roche Diagnostics (Roche Diagnostics GmbH). Seventy-one (71.4%) of the cycles occurred prior to January 2018, and the remaining 28.6% after this date.

Primary outcome measures included ovarian response to each OI regimen.

Statistics

For each treatment type, differences in serum AMH levels in cycles with response to treatment versus cycles with no response were evaluated using p values that were generated from a mixed effects logistic regression model of AMH on treatment response, accounting for patients contributing more than one cycle to any particular treatment type. The following AMH percentiles (pct.), derived from our total study population (25th (5.4 ng/ml), 50th (9.3 ng/ml), 75th (14.0 ng/ml), 90th (19.0 ng/ml)), were used as cutoffs to determine the predicted probabilities (PProb) of response to treatment. For the latter, we used mixed effects logistic regression modeling, adjusting for age and BMI, and controlling for the potential of more than one cycle per woman. Since gonadotropin cycles were characterized by an almost universal response to treatment, it was impossible to calculate PProb and odds ratio (OR) for treatment response among patients in this group. The level of statistical significance was set at 0.05. All statistical analyses were performed in Stata version 14.0.

Results

Ovulation induction cycles

Ovulation induction cycles from 2003 to 2019 were reviewed for response to treatment. Two thousand three hundred and nine (2309) OI cycles were identified among PCOS patients, but pre-treatment AMH information was available in 738 cycles only (derived from 242 women). Of those in 295 (40.0%), 180 (24.4%), and 263 (35.6%) cycles, either CC, LET, or FSH were used, respectively. In 59.7% (157/263) of FSH cycles, prior treatment with oral agents had been used. For the FSH group, the average (SD) cycle length was 17.3 (6.2) days, and the average (SD) daily dose was 63.0 (30.6) IU. The demographic and cycle characteristics of the study population are shown in Table 1. Age and BMI did not differ significantly among the treatment groups, whereas serum AMH levels did ($p = 0.04$) (Table 1).

Treatment response and AMH

Overall, the majority of cycles (660 cycles (89.4%)) demonstrated a response to treatment. In the oral medication cycles, where no response was noted to the initial treatment dose, the dose was increased in a subsequent attempt in 60% of the cycles, and the same OI agent was utilized. In 80% of those, response to treatment was observed. In 15% of the cycles with no response, gonadotropins were utilized in a subsequent attempt, and in all but one cycles response to treatment was observed. Finally, in the remaining oral medication cycles with no response to treatment, either a different oral agent was utilized in a subsequent cycle or the same agent was used without altering the dose, and overall response was observed in 78.6% of them.

When the response rates were compared among the three treatment groups, significant differences were observed ($p < 0.001$). Nearly all FSH cycles (98.5%) responded to treatment, while lower response rates were noted among CC (87.8%) and LET (78.9%) cycles. In our total population, cycles characterized by response to treatment when compared to those with no response had significantly lower AMH levels. This difference persisted even when the comparison was limited within each of the oral OI regimen groups, where cycles with treatment response had lower AMH levels than the ones with no response (for both CC and LET groups). The difference in AMH levels was attenuated within the FSH group (Table 2).

When cycles were stratified by serum AMH pct., all of those with serum AMH ≤ 10th pct. (3.1 ng/ml) were characterized by response to medication. Overall, after adjusting for age and BMI, there was a trend for lower PProb for response to treatment with higher serum AMH levels ($p < 0.001$) (Table 3). Similarly, this trend persisted when analysis was restricted within each treatment group for CC and LET cycles ($p < 0.001$ and $p = 0.075$, respectively) (Table 3), but response to OI was noted in nearly all FSH cycles regardless of serum AMH levels.

Overall, cycles with AMH ≤ 25th pct. (5.4 ng/ml) were almost 6 times more likely to respond to any treatment over the ones with AMH > 25th pct. (OR 5.7, 95%CI 2.1–15.5, $p < 0.001$). Similarly, when limiting the analysis in CC cycles only, the odds of treatment response was 13.5 times higher in cycles with AMH ≤ 25th pct. compared to cycles with AMH > 25th pct. (OR 13.5, 95%CI 2.0–92.1, $p = 0.01$), whereas cycles with AMH ≥ 75th pct. (14.0 ng/ml) had significantly lower odds to respond to CC over those with AMH < 75th pct. (OR 0.2, 95%CI 0.04–0.8, $p = 0.02$). Although this trend was maintained in LET cycles as well, the difference did not reach statistical significance: neither for LET cycles with AMH ≤ 25th pct. compared to cycles with AMH > 25th pct. (OR 5.6, 95%CI 0.7–43.4, $p = 0.10$) nor for those with AMH ≥ 75th pct. compared to cycles with AMH < 75th pct. (OR 0.6, 95%CI 0.3–1.4, $p = 0.25$) (Fig. 1).

Table 1 Baseline characteristics of the study population stratified by ovulation induction medication, described by cycle

	All cycles (n = 738)	CC (n = 295)	LET (n = 180)	FSH (n = 263)
Age, years				
Median (IQR)	32.3 (30.8–34.7)	32.5 (30.8–34.8)	32.1 (30.5–34.2)	32.7 (31.0–34.8)
AMH, ng/ml				
Median (IQR)	9.3 (5.4–14.0)	7.6 (4.5–13.0)	11.0 (7.5–14.0)	10.0 (5.9–14.0)
Percentiles				
10th	3.1	2.9	4.5	2.8
25th	5.4	4.5	7.5	5.9
75th	14.0	13.0	14.0	14.0
90th	19.0	19.6	19.0	19.0
Day 3 FSH, U/L				
Median (IQR)	5.8 (4.8–6.7)	6.0 (5.1–6.7)	5.8 (4.5–6.8)	5.5 (4.6–6.8)
BMI, kg/m^2				
Median (IQR)	24.6 (22.1–30)	24.6 (22.3–29.4)	23.5 (21.8–30.6)	25.7 (22.1–31.1)
Race, n (%)				
Caucasian	463 (62.7)	201 (68.1)	117 (65.0)	145 (55.1)
Asian	105 (14.2)	25 (8.5)	26 (14.4)	54 (20.5)
Hispanic	44 (6.0)	19 (6.4)	11 (6.1)	14 (5.3)
African American	37 (5.0)	16 (5.4)	6 (3.3)	15 (5.7)
Indian	31 (4.2)	6 (2.1)	3 (1.7)	22 (8.4)
Mixed	44 (6.0)	16 (5.4)	16 (8.9)	12 (4.6)
Unknown	14 (1.9)	12 (4.1)	1 (0.6)	1 (0.4)
No. of follicles > 13 mm[a]				
Median (IQR)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.0)	1.0 (1.0–2.0)
Endometrial thickness, (mm)[a]				
Median (IQR)	8.0 (6.0–9.2)	7.3 (6.0–9.0)	6.9 (5.6–8.0)	9.0 (7.3–10.4)

CC clomiphene citrate, *LET* letrozole, *FSH* follicle-stimulating hormone, *AMH* anti-Müllerian hormone, *BMI* body mass index. [a] These values were recorded on the last monitoring ultrasound prior to trigger

Discussion

Ovulation induction is often the first line of treatment among infertile women with PCOS and the selection of the proper medication is important in achieving ovulation and ultimately pregnancy in such patients. Personalized medicine is still emerging in reproductive sciences, although it has advanced immensely in other areas, such as oncology. New biomarkers are being described and the significance of already established markers is reassessed [33]. Given the availability of multiple regimens for OI, tailored patient-specific ovarian stimulation protocols may allow for optimal treatment response rates.

Table 2 Response to treatment in relation to serum anti-Müllerian hormone (AMH) levels, described by cycle

	Overall treatment		Clomiphene citrate		Letrozole		FSH	
AMH (ng/ml)	No-RT (n = 78)	RT (n = 660)	No-RT (n = 36)	RT (n = 259)	No-RT (n = 38)	RT (n = 142)	No-RT (n = 4)	RT (n = 259)
Mean (SD)	13.7 (7.1)	10.1 (6.6)	13.8 (7.9)	9.2 (7.2)	13.1 (6.0)	10.7 (5.4)	18.3 (9.3)	10.7 (6.4)
Median (IQR)	12.0 (8.6–19.0)	8.8 (5.0–13.0)	11.5 (7.8–19.0)	7.3 (3.9–12.0)	11.7 (8.7–15.0)	11.0 (6.8–14.0)	16.5 (10.5–28.0)	10.0 (5.8–14.0)
p value*	< 0.001		0.017		0.019		< 0.001**	

No-RT no response to treatment, *RT* response to treatment, *FSH* follicle-stimulating hormone

*p values generated from mixed effects logistic regression of AMH on treatment response for each treatment type, to account for patients contributing more than one cycle to any particular treatment type

**For the FSH group, p value results should be interpreted with extreme caution since 98.5% of cycles showed response to treatment and only 4 cycles comprise the no-RT group

Table 3 Predicted probability of response to treatment (PProb-RT)

AMH percentile (pct.) (ng/ml)	All treatments (N = 738) PProb-RT (95%CI)	Clomiphene citrate (N = 295) PProb-RT (95%CI)	Letrozole (N = 180) PProb-RT (95%CI)
≤ 25th pct. (5.4)	97% (95–99%)	97% (93–100%)	95% (85–105%)
≥ 50th pct. (9.3)	87% (83–90%)	79% (70–88%)	75% (67–83%)
≥ 75th pct. (14.0)	85% (79–90%	75% (61–89%)	73% (60–86%)
≥ 90th pct. (19.0)	82% (73–90%)	75% (58–92%)	69% (49–90%)
P for trend	< 0.001	< 0.001	0.075

AMH anti-Müllerian hormone. Reported results are adjusted for mean age and body mass index of the study population

Our data suggest that among subpopulations of PCOS patients with high serum AMH levels and therefore a higher probability of no response to orally administered ovarian OI agents, there might be a benefit to using gonadotropins as a first-line OI agent (while utilizing a conservative approach to stimulation) or to considering a higher initial dose for the oral agent. In our cohort, nearly all cycles with gonadotropins achieved ovulation regardless of AMH, while significantly lower response rates were noted among cycles with AMH above the 75th or 90th pct. (14.0 or 19.0 ng/ml, respectively) of our population utilizing oral OI agents.

As seen in other studies, serum AMH was significantly lower in cycles with response to CC and LET compared to cycles with no response to these agents [26, 30, 34]. The effect of AMH on outcomes of ovulation induction with gonadotropins is not very well described. Amer et al. [35] and Di Paola et al. [36] suggested that PCOS women with substantially elevated serum AMH levels would have a diminished response to ovulation induction with gonadotropins and recommended stimulation with increased dosage, which, however, might ultimately lead to over-response. Nevertheless, the restricted number of participants (*n* = 20 and *n* = 22 PCOS patients, respectively) and the lack of additional information concerning response rates after using increased doses limit the generalizability of these findings. With our population of 263 FSH cycles in PCOS patients and the consistency in the

management of patients undergoing ovarian stimulation, the present study adds novel information to the existing literature.

Women with PCOS typically have elevated serum AMH levels, potentially due to the increased number of small antral follicles, the higher AMH production per granulosa cell, and/or a positive correlation with androgens [37]. AMH appears to inhibit the gonadotropin-induced aromatase expression in granulosa cells and, also, to reduce the FSH receptor mRNA expression [38]. While for low AMH levels the probability of response to CC or LET was excellent, our analysis showed that this probability decreased as AMH increased. In our study population, the probability of no response to treatment (for either CC or LET) was over 20% for cycles with serum AMH levels above the median of our population (9.3 ng/ml). In these patients, it might be preferable to skip OI with oral agents and proceed to a conservative gonadotropin stimulation regimen instead, since response to treatment was achieved in nearly all cycles in which FSH was used, while the number of generated pre-ovulatory follicles remained comparable between all groups. We could hypothesize that the effect of CC or LET in the endogenous pathway of gonadotropin production may not be enough to overcome the inhibitory effect of AMH when serum levels are above a certain point. As for exogenous gonadotropin administration, a step-up dosing protocol or increased duration of treatment might outweigh the negative effect of AMH. However, among women with substantially elevated serum AMH values, gonadotropin
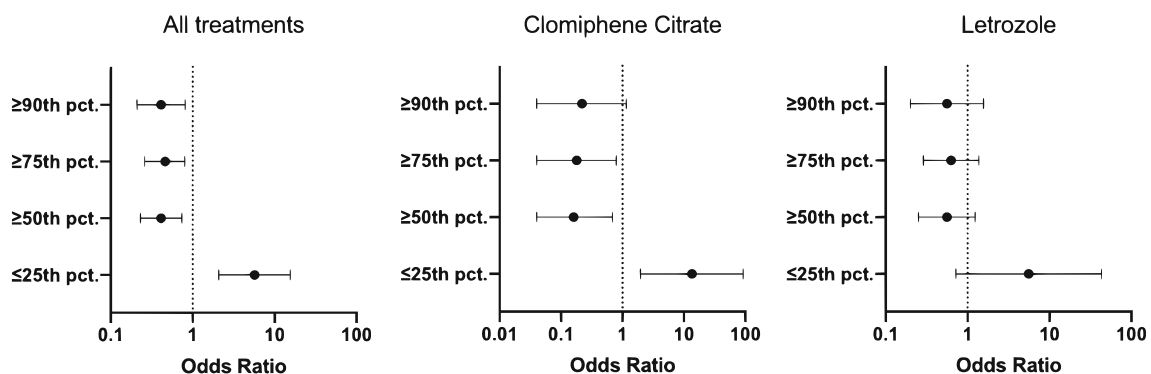

Fig. 1 Association between anti-Müllerian hormone levels (expressed in percentiles, pct.) and treatment response (odds ratio, 95% confidence intervals) for the different treatment groups, after adjusting for mean age and body mass index of the study population

stimulation requires careful selection of the dose, and closer monitoring with the ultimate goal of mono-ovulatory response, so as to decrease the risk of multiples and ovarian hyperstimulation syndrome, given the positive correlation reported for the latter with serum AMH levels in the in vitro fertilization (IVF) setting [39]. Alternatively, if oral agents are preferred by either the treating physician or the couple, it might be reasonable to consider a higher starting dose.

Our study has a few strengths. Our PCOS population is diverse and includes patients in all BMI and AMH groups. All patients were evaluated and treated in a single academic practice utilizing the same clearly defined protocols. Furthermore, the fact that we did not focus on absolute AMH values but rather on AMH percentiles, as defined by our population, increases the generalizability of our findings, since it might partially account for the variability of the AMH assays between clinics. Our study has certain limitations, as well. A new assay for the measurement of AMH was used towards the later part of the study (affecting 28.6% of the cycles). The performance of the assays and their degree of agreement have been previously evaluated with results suggesting that they provide comparable measurements with a slight discordance mainly in the lower values [40]. Since the vast majority of our patients do not have AMH levels in the low range, where the assays slightly differ, the impact should be minimal if any. The 10th pct. for AMH in our study population is at 3.1 ng/ml; therefore, very few patients, if any, fall in the low range where the assays might differ in performance. Furthermore, the utilization of AMH percentiles, rather than absolute values, might account further for any variability in the assays. Interestingly, the mean (SD) BMI of our population (26.5 (5.7) kg/m^2) is lower than that reported among other PCOS cohorts, and the interquartile range was 22.1–30.0 kg/m^2. The Fertility Center utilizes a strict BMI cutoff of 40.0 kg/m^2 to initiate treatments and this might have contributed to the observed difference. However, given the association between BMI and response to treatment [32], one would not expect patients with BMIs higher than that of our population to respond better. For that reason, one can assume that our leaner population represents the "best-case scenario" regarding the possibility of response. Notwithstanding, approximately 40% of the cycles in the current study derive from obese PCOS patients. Finally, the study is limited by its retrospective design.

In conclusion, we showed that, for patients with PCOS, higher serum AMH levels, as compared to lower levels, are associated with significantly lower probability of response to either CC or LET, whereas nearly all patients responded to FSH irrespective of pre-treatment AMH levels. With AMH values ≤ 25th pct., the vast majority of patients would respond to either treatment, while with AMH values > 75th percentile (> 14.0 ng/ml in our study population) the probability of response decreased for both CC and LET cycles. Our findings constitute a valuable tool for the clinician when counseling PCOS patients and designing a personalized ovulation induction treatment strategy.

Declarations

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