Testosterone and luteinizing hormone predict semen parameter improvement in infertile men treated with anastrozole

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Objective: To identify patient factors associated with a clinically significant improvement in semen parameters among infertile men treated with the aromatase inhibitor anastrozole.
Design: Multi-institutional retrospective cohort study.
Setting: Two Tertiary Academic Medical Centers.
Patients: A total of 90 infertile men treated at 2 tertiary academic medical centers who met inclusion criteria and obtained pretreatment and posttreatment semen analyses.
Intervention: Prescription of anastrozole (median 3 mg/wk).
Main Outcome Measures: Upgrade in the World Health Organization sperm concentration category (WHO-SCC). Univariate logistic regression, multivariable logistic regression, and partitioning analyses were performed to identify statistically significant patient factors capable of predicting treatment response.
Results: With anastrozole treatment, 46% (n = 41/90) of men responded favorably with a WHO-SCC upgrade, and 12% (n = 11/90) experienced a downgrade. Responders exhibited lower pretreatment levels of luteinizing hormone (LH, 4.7 vs. 8.3 IU/L) and follicle-stimulating hormone (4.7 vs. 6.7 IU/mL), higher pretreatment levels of testosterone (T, 356 vs. 265 ng/dL), and similar baseline level of estradiol (E_2, 73% vs. 70% with detectible level). Baseline semen parameters differed, with anastrozole responders demonstrating higher baseline semen concentration (3.6 vs. 0.3 M/mL) and higher total motile sperm counts (3.7 vs. 0.1 M). Anastrozole therapy converted 29% (n = 26/90) of the cohort to normozoospermia and enabled intrauterine insemination access in 31% (n = 20/64) of previously ineligible patients. Interestingly, neither body mass index nor the baseline E_2 level or E_2-T ratio was associated with WHO-SCC upgrade. Multivariable logistic regression revealed the T-LH ratio (odds ratio: 1.02, 95% confidence interval: 1.00–1.03) and baseline nonazoospermia (odds ratio: 9.4, 95% confidence interval: 1.1–78.9) to be statistically significant predictors of WHO-SCC upgrade (area under receiver operating characteristic curve: 0.77). The final user-friendly partitioning model consisting of the T-LH ratio $\geq$100 and baseline non-azoospermia was 98% sensitive and 33% specific for WHO-SCC upgrades (area under the curve: 0.77).
Conclusion: Anastrozole therapy decreases serum E_2 levels, increases serum gonadotropins, and clinically improves semen parameters in half of men with idiopathic infertility. Nonazoospermic infertile men with T-LH ratios $\geq$100 are likely to benefit from anastrozole treatment irrespective of baseline E_2 level or E_2-T ratio. Men with azoospermia rarely respond to anastrozole and should be counseled on alternative treatments. (Fertil Steril® 2023;120:746–54. ©2023 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Anastrozole, aromatase inhibitor, semen analysis, male infertility, gonadotropins

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Approximately 15% of couples worldwide experience infertility, defined as the inability to achieve pregnancy after 12 months of regular, unprotected sexual intercourse (1). Despite the presence of a male factor in nearly half of the infertile couples, 40%–70% of patients with impaired semen parameters have no identifiable underlying etiology (2, 3). Empiric medical treatment for idiopathic male infertility aims to augment spermatogenesis via modulation of the hypothalamic-pituitary-gonadal hormone axis, typically through the utilization of selective estrogen receptor modulators, aromatase inhibitors (AIs), and/or exogenous gonadotropins (Gns) (4–6). Aromatase inhibitors such as anastrozole limit the conversion of testosterone (T) to estradiol (E_2), minimize negative inhibition on pituitary access, and increase endogenous Gn production (2, 7).

Aromatase inhibitors have been offered historically to patients with a T-E_2 ratio <10 (8, 9). Anastrozole, a more selective inhibitor than prior agents, has been shown to decrease serum E_2 levels and improve semen parameters in men with a low T-E_2 ratio (10, 11). A recent meta-analysis found that the use of AIs was associated with improved sperm concentrations and endocrine profiles (12). Anastrozole may also benefit those also with morbid obesity who express aromatase at higher levels (13).

Although anastrozole has increasingly been employed to treat infertile men with elevated peripheral estrogen, it is unclear whether a broader cohort may benefit also from therapy. In this retrospective multi-institutional cohort study, we aimed to identify baseline patient factors that predict a clinically significant response to anastrozole among men with impaired semen parameters. We hypothesized that non-azoospermic men with high E_2 levels and low Gn levels would be most likely to experience a clinically meaningful response, defined by an increase in the World Health Organization sperm concentration category (WHO-SCC) (14). Our goal was to identify predictors of clinically significant treatment response and develop a simple algorithm to guide the initiation of anastrozole among infertile men.

MATERIALS AND METHODS

Cohort Construction and Data Collection

Institutional review board approval (Cleveland Clinic [CCF]-IRB 19-212, University of California Los Angeles [UCLA]-IRB 20-000710) was obtained, and the electronic medical record at each institution was queried to identify infertile men treated with anastrozole (CCF: 07/2009–01/2021, UCLA: 04/2016–07/2021). Demographics, hormone parameters, semen parameters, and fertility interventions were captured from the medical record. Exclusion criteria included pretreatment normozoospermia (>15 M/mL), exogenous T use within 6 months of initiating therapy, prior orchiectomy, scrotal surgery during the treatment period, pretreatment semen analysis (SA) collected >1 year before anastrozole prescription, and/or posttreatment SA collected <1 month after initiating therapy. Laboratory studies and semen analyses were completed at the institution where patients sought care. Both institutions used electrochemiluminescence immunoassays to quantify T levels.

Given the objective of identifying predictors of a clinically significant response, patients were designated as anastrozole responders or nonresponders on the basis of an upgrade in WHO-SCC. World Health Organization sperm concentration categories include azoospermia, cryptozoospermia (sperm identified within a pelleted specimen), severe oligozoospermia (<5 M/mL), oligozoospermia (5–15 M/mL), and normozoospermia (>15 million/mL) (14). This metric has been previously used to predict treatment response among infertile men receiving empiric medical treatment (15).

Data Analysis

Descriptive statistics were completed to characterize the multi-institutional cohort. Normality was assessed by qualitatively assessing the distribution of each variable on histograms and quantile-quantile plots. Wilcoxon rank-sum tests were used to compare continuous variables across groups. χ^2 and Fisher's exact tests were used to compare categorical variables. Paired *t* tests were applied to compare pretreatment and posttreatment levels for continuous variables. Semen parameters included an excess of zero-value differences, so dependent statistical testing was not performed. Statistical analyses were completed with the use of JMP 16.0.

Anastrozole dosing was not standardized, and thus the median dose and interquartile range (IQR) were reported when baseline patient characteristics were considered. Total motile sperm counts (TMSCs) were calculated as the product of sperm concentration, semen volume, and motility percentage. A sperm motility percentage was not recorded for patients with baseline azoospermia or cryptozoospermia. Estradiol levels were handled also with caution: Estradiol assays used by each institution differed with regard to the lowest level of detectable hormone (CCF: 25 pg/mL, UCLA: 12 pg/mL). Therefore, baseline E_2 concentration was treated as a binary categorical variable with a cutoff set at $\geq$25 pg/mL. Although the T-E_2 ratio has classically been reported in fertility literature, we considered E_2 <25 pg/mL to be null and reported the inverse ratio (E_2-T), so this parameter would remain a rational quantitative number for all hormone values.

Predictor Variable Selection

We aimed to identify patient factors associated with WHO-SCC upgrades that would be available to the clinician when anastrozole initiation is considered. Baseline clinical features, infertility interventions, semen parameters, and hormone levels were evaluated. The performance of each candidate variable was assessed with univariate logistic regression modeling, partitioning, and bootstrap forest analysis. The 6 best-performing variables were noted for each test. Variables were considered for predictive modeling when selected by 2 or more screening methods.

Predictive Modeling

Multivariable logistic regression modeling was performed using combinations of selected predictor variables. High-performance models were reported with the corresponding area under the curve (AUC) statistic and the specificity

associated with the cutoff point achieving 95% sensitivity. Odds ratios, confidence intervals, and effect-test significance values were provided for each covariate. The best-performing multivariable model was subjected to recursive partitioning, with cutoffs informed by the partitioning algorithm (16). A sensitivity analysis was performed to compare the predictive power of the E_2-T ratio to the partition model.

RESULTS

Effects of Anastrozole Therapy

Among the 90 infertile men in the final cohort, the median age was 36 years (IQR: 32–41) and the median body mass index (BMI) was 32 kg/m^2 (IQR: 27–43 kg/m^2, Table 1). Anastrozole was typically prescribed at 3 mg per week (IQR: 3–7 mg/wk, Supplemental Fig. 1, available online). The median time between pretreatment SA and initiation of anastrozole was 18 days (IQR: 0–49 days), whereas the median time between anastrozole treatment and posttreatment SA was 91 days (IQR: 64–117 days, Supplemental Fig. 2, available online). After initiation of anastrozole at any dose, E_2 was suppressed to undetectable levels in most patients (n = 60/76, 79%, Supplemental Table 1, available online). Serum levels of luteinizing hormone (LH, 8.5 vs. 6.4 IU/L), follicle-stimulating hormone (FSH, 8.5 vs. 5.6 IU/mL), and T (469 vs. 295 ng/dL) all increased after aromatase inhibition. Among all patients studied, median sperm concentration (4.4 vs. 2.3 M/mL) and TMSC (3.5 vs. 0.9 M) increased after aromatase inhibition. Posttreatment sperm motility (42% vs. 36%) modestly improved, whereas semen volume (2.5 vs. 2.5 mL) was similar to pretreatment values.

Characteristics of Anastrozole Responders and Nonresponders

Approximately half (n = 41/90, 46%) of men experienced a WHO-SCC upgrade after treatment with anastrozole (Fig. 1A). Responders and nonresponders (Table 1) were similar with regard to age (37 vs. 36 years, P=.63), BMI (33 vs. 32 kg/m^2, P=.98), and smoking history (32 vs. 33%, P=.92). Responders possessed incrementally greater median total testicular volume than nonresponders (34 vs. 30 mL, P=.005), although both groups had a similar prevalence of varicocele (50 vs. 40%, P=.39). Responders exhibited lower pretreatment LH (4.7 vs. 8.3 IU/L, P=.0002) and FSH (4.7 vs. 6.7 IU/mL, P=0.01), higher baseline T (356 vs. 265 ng/dL, P=.08), and comparable detectible pretreatment E_2 (73 vs. 70%, P=.81). Anastrozole dose was not associated with responder status (P=.49, Supplemental Fig. 1), and patients on higher weekly

TABLE 1

Baseline characteristics of men with idiopathic infertility treated with anastrozole.

Variable[a]	No. missing	Overall (n = 90)	Responders (n = 41)	Nonresponders (n = 49)	*P* value[b]
Clinical features before treatment					
Age (y)	0	36 (32–41)	37 (31–41)	36 (33–40)	.63
BMI (kg/m^2)	3	32 (27–43)	33 (27–43)	32 (27–43)	.98
Smoking history	0	29 (32%)	13 (32%)	16 (33%)	.92
Genitourinary surgery history	0	16 (18%)	4 (10%)	12 (24%)	.10
Total testicular volume (cc)	3	30 (24–36)	34 (28–40)	30 (24–34)	**.005**
Varicocele history	2	39 (44%)	20 (50%)	19 (40%)	.39
Medical management of infertility					
Anastrozole dose (mg/wk)	0	3 (3–7)	3 (3–7)	3 (3–5)	.49
Anastrozole dose > 3 mg/wk	0	26 (29%)	14 (34%)	12 (24%)	.31
Clomiphene citrate prescription history	0	25 (28%)	13 (32%)	12 (24%)	.49
hCG prescription history	0	8 (9%)	6 (15%)	2 (4%)	.13
Semen parameters before treatment					
Semen volume (mL)	1	2.5 (1.6–3.4)	2 (1.3–2.9)	2.8 (1.8–3.6)	**.01**
Sperm concentration (M/mL)	0	2.3 (0.0–6.8)	3.6 (0.3–8.1)	0.3 (0.0–5.4)	**.01**
Percent motile (%)[c]	0	36 (26–47)	38 (25–50)	35 (29–43)	.42
Total motile sperm count (M)	0	0.9 (0.0–5.9)	3.7 (0.1–6.2)	0.1 (0.0–5.0)	**.04**
Baseline WHO semen classification category					
Azoospermia	0	19 (21%)	3 (7%)	16 (33%)	**.02**
Cryptozoospermia	0	11 (12%)	5 (12%)	6 (12%)	
Severe oligozoospermia	0	32 (36%)	17 (41%)	15 (31%)	
Oligozoospermia	0	28 (31%)	16 (39%)	12 (24%)	
Hormone parameters before treatment					
LH (IU/L)	1	6.4 (3.8–11.7)	4.7 (3.2–6.7)	8.3 (5.4–12.9)	**.0002**
FSH (IU/mL)	0	5.6 (3.9–10.5)	4.7 (3.1–7.5)	6.7 (4.6–12.7)	**.01**
Testosterone (ng/dL)	0	295 (190–440)	356 (196–487)	265 (178–362)	.08
Estradiol ≥25 pg/mL[d]	5	61 (72%)	30 (73%)	31 (70%)	.81

Bolded values represent $P < .05$.
Abbreviations: BMI = body mass index; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; LH = luteinizing hormone; WHO = World Health Organization.
[a] Categorical variables were described with counts and percentages of the column total (adjusted for missing data). Continuous variables were described with a median value and an interquartile range.
[b] Categorical variables were compared using a chi-squared or Fisher Exact test. Wilcoxon rank-sum tests were used to compare continuous variables across groups.
[c] Motility percentages were not recorded for patients with azoospermia and cryptozoospermia.
[d] The estradiol assays used by each institution differed with regard to the lowest level of detectable hormone. Therefore, this variable was reported as a categorical variable, with the higher of the 2 detection thresholds being used as the cutoff.

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doses of anastrozole (>3 mg/wk) were not more likely to experience a WHO-SCC upgrade (34 vs. 24%, $P=.31$).

Baseline semen parameters also differed between groups, with anastrozole responders exhibiting a higher baseline sperm concentration (3.6 vs. 0.3 M/mL, $P=.01$) and a higher TMSC (3.7 vs. 0.1 M, $P=.04$) (Table 1). Severe oligozoospermia (n = 32/90, 36%) and oligozoospermia (n = 28/90, 31%) were the most common pretreatment WHO-SCCs for the entire cohort and for anastrozole responders. Azoospermia was the most common baseline WHO-SCC among nonresponders (n = 16/49, 33%) and was responsible primarily for the significant difference in pretreatment WHO-SCC categorization ($P=.02$). Although 46% (n = 41/90) of men experienced a WHO-SCC upgrade after initiating anastrozole, 42% (n = 38/90) remained in the same WHO-SCC group. Importantly, 12% (n = 11/90) experienced a downgrade after treatment (Fig. 1A). A minority of the cohort was concomitantly prescribed clomiphene citrate (CC, n = 21/90, 23%) or human chorionic gonadotropin (hCG, n = 6/90, 7%, Supplemental Table 5). There was no statistically significant difference between anastrozole responder status with respect to CC or hCG prescription history ($P=.49$ and $P=.13$, respectively).

Despite achieving similar rates of E_2 suppression (78% vs. 80%), posttreatment levels of LH (5.9 vs. 12.7 IU/L, $P<.0001$) and FSH (7.4 vs. 9.4 IU/mL, $P<.0001$) were lower among anastrozole responders, whereas serum T levels (488 vs. 406 ng/dL, $P=.03$) remained marginally higher. The median posttreatment sperm concentrations (23.0 vs. 3.6 M/mL) and TMSCs (19.3 vs. 3.7 M) were higher among responders than nonresponders (Table 1).

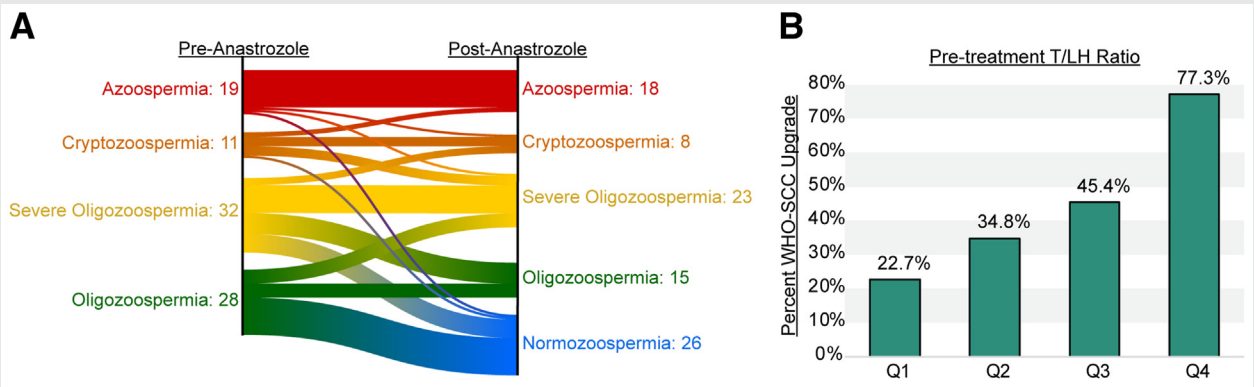
Serum Hormone Ratios

Given clear differences in pretreatment Gn levels, we evaluated the utility of hormone ratios in predicting anastrozole responder status. We found that the baseline T-LH ratio was significantly elevated among those who experienced a WHO-SCC upgrade (75 vs. 38, $P=.0002$). The predictive value of the T and LH variables was further illustrated by evaluating hormone and ratio performance using quartile (Q) (Fig. 1B). Men with the lowest Qs of baseline LH were most likely to experience a WHO-SCC upgrade (Q1: 70.8%, Q2: 57.1%), whereas those with the highest Qs of pretreatment T levels were most likely to respond (Q3: 52.2%, Q4: 57.1%) (Supplemental Fig. 3, available online). This finding is further accentuated across Qs of the baseline T and LH variables, with 77.3% of the highest Q4 achieving a WHO-SCC upgrade (Fig. 1B).

Identification of Predictor Variables

We then used univariate logistic regression modeling to identify patient factors able to predict a clinically significant response to anastrozole among infertile men. Anastrozole dosing was not strongly associated with responder status (AUC: 0.546, $P=.50$). The remaining predictor variables were grouped into 3 categories: physical examination (PE) findings, SA results, and Gn levels. Although several baseline clinical histories and PE features were screened, only total testicular volume was significantly associated with the WHO-SCC upgrade (AUC: 0.676, $P=0.0004$, Table 2). The T-LH ratio was the best-performing variable overall, with the highest AUC statistic (AUC: 0.744) and lowest effect likelihood ratio P value ($P<.0001$). The T-LH ratio predicted WHO-SCC upgrade better than either pretreatment T (AUC: 0.609, $P=.03$) or LH (AUC: 0.731, $P=.0003$) alone. Multiple semen parameters were strongly associated with a clinically significant response to anastrozole therapy, with baseline nonazoospermia (AUC: 0.627, $P=.002$), sperm concentration (AUC: 0.655, $P=.02$), and semen volume (AUC: 0.651, $P=.02$) showing the highest predictive value.

FIGURE 1



The testosterone-to-luteinizing (T-LH) hormone ratio predicts response to anastrozole treatment, according to an upgrade in the World Health Organization sperm classification category (WHO-SCC). (A) 46% (n = 41/90) of the multi-institutional cohort experienced a WHO-SCC upgrade after initiating anastrozole therapy. Nonresponders included the 42% (n = 38/90) of the cohort that remained in the same WHO-SCC group and the 12% (n = 11/90) of the cohort who experienced a downgrade after the treatment. (B) Higher levels of the T-LH ratio identify those with a clinically significant response to anastrozole, with the highest quartile (Q4) being associated with a 77.3% incidence of WHO-SCC upgrade.

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TABLE 2

Performance of predictor variables in univariate logistic regression models of the World Health Organization sperm concentration category upgrade.

Variable	Odds Ratio	95% CI	*P* value[a]	AUC[b]
Baseline clinical features				
Age (y)	1.025	0.960–1.096	.47	0.530
BMI (kg/m2)	1.004	0.967–1.043	.83	0.501
Smoking history	0.958	0.394–2.328	.92	0.505
Genitourinary surgery history	0.333	0.098–1.129	.06	0.574
Total testicular volume (mL)	1.074	1.019–1.132	**.004**	0.676
Varicocele history	1.526	0.654–5.563	.33	0.552
Institution (UCLA)	1.64	0.705–3.815	.25	0.560
Medical management of infertility				
Anastrozole dose (mg/wk)	1.073	0.875–1.319	.50	0.546
Anastrozole dose >3 mg/wk	1.599	0.640–4.053	.31	0.548
Clomiphene citrate prescription history	1.432	0.567–3.612	.45	0.536
hCG prescription history	4.027	0.767–21.14	.08	0.553
Semen parameters before treatment				
Semen volume (mL)	0.631	0.461–0.925	**.02**	0.651
Sperm concentration (M/mL)	1.121	1.012–1.246	**.02**	0.655
Percent motile (%)	1.014	0.983–1.045	0.39	0.560
Total motile sperm count (M)	1.040	0.964–1.121	0.31	0.626
Nonazoospermia[c]	6.141	1.643–22.95	**.002**	0.627
Hormone parameters before treatment				
LH (IU/L)	0.865	0.783–0.954	**.0003**	0.731
FSH (IU/mL)	0.918	0.849–0.991	**.01**	0.657
Testosterone (ng/dL)	1.003	1.000–1.005	**.03**	0.609
Estradiol ≥25 pg/mL[d]	1.144	0.444–2.948	.78	0.514
Hormone ratios before treatment				
E2-T ratio	2.667	0.075–94.84	.59	0.523
T-LH ratio	1.020	1.008–1.033	**<.0001**	0.744

Bolded values represent $P < .05$.
Abbreviations: AUC = area under the curve; BMI = body mass index; CI = confidence interval; E_2 = estradiol; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; LH = luteinizing hormone; T = testosterone; UCLA = University of California Los Angeles.
[a] Categorical variables were described with counts and percentages of the column total (adjusted for missing data). Continuous variables were described with a median value and an interquartile range.
[b] Categorical variables were compared using a chi-squared or Fisher Exact test. Wilcoxon rank-sum tests were used to compare continuous variables across groups.
[c] Motility percentages were not recorded for patients with azoospermia and cryptozoospermia.
[d] The estradiol assays used by each institution differed with regard to the lowest level of detectable hormone. Therefore, this variable was reported as a categorical variable, with the higher of the 2 detection thresholds being used as the cutoff.

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Predictor variables were further subjected to partition and bootstrap forest analyses to assess predictive power (Supplemental Table 2, available online). Six predictor variables were identified as top performers across 2 of the 3 screening methods. Variables selected for consideration in multivariable models included total testicular volume, semen volume, sperm concentration, baseline nonazoospermia, pretreatment LH, and baseline T-LH ratio.

Multivariable Model Construction and Performance

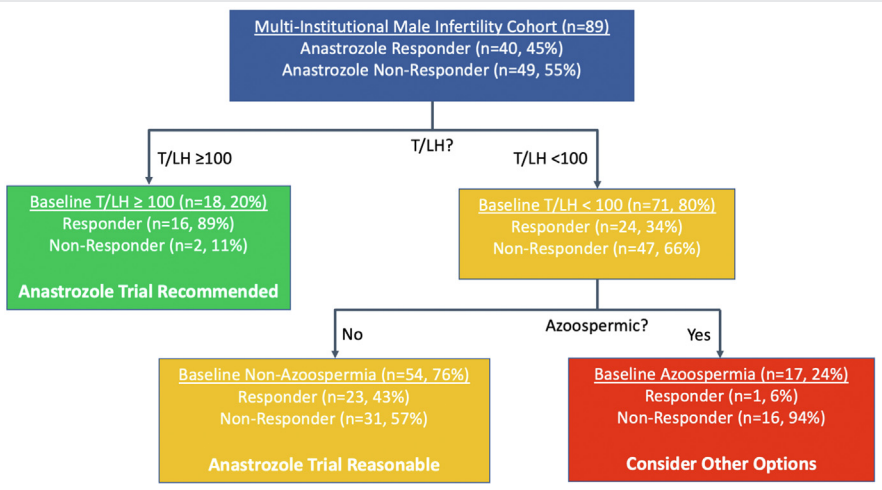
To identify the patients most likely to benefit from anastrozole treatment, we generated multivariable models prioritizing sensitivity rather than specificity for the WHO-SCC upgrade. Top-performing models representing combinations of the 3 predictor categories (PE, SA, and Gn) were reported because these represent clinically useful models applicable during clinical evaluation (Supplemental Table 3, available online).

Using the probability cutoff associated with 95% sensitivity (i.e., 95% of responders are above the cutoff), the univariate Gn model (T/LH alone) achieved a specificity of 29% (i.e., 29% of nonresponders remained below the cutoff) (AUC: 0.74). At this same sensitivity threshold, the specificity of the multivariable Gn + PE model (T/LH and total testicular volume as covariates) increased to 38% with a marginal improvement in predictive value (AUC: 0.75). The multivariable Gn + SA model (T/LH and baseline nonazoospermia) performed better yet, achieving 44% specificity and enhanced predictive value (AUC: 0.77). Notably, both covariates in the Gn + SA model significantly contributed to model performance (T-LH: $P<.0001$, baseline nonazoospermia: $P=.0009$). The more complex Gn + SA + PE model performed incrementally better with 49% specificity (AUC: 0.78), although total testicular volume did not significantly contribute to model performance ($P=.34$).

Partition Analysis

Recursive partitioning was used to generate a simplistic clinical model, in which high-performing predictor variables discriminate between anastrozole responders and nonresponders (Fig. 2). The T-LH ratio was the best initial differentiator at a cutoff value of ≥100, the T-LH ratio selected 18 patients, 16 (89%) of whom experienced a clinically significant response to anastrozole treatment. The remaining

FIGURE 2



The testosterone-to-luteinizing hormone (T-LH) ratio and baseline nonazoospermia identify appropriate candidates for the initiation of anastrozole. Recursive partitioning analysis identified the baseline T-LH ratio with a cutoff ≥100 as the best initial differentiator for selecting patients most likely to experience a World Health Organization sperm classification category (WHO-SCC) upgrade. The cohort was further partitioned by identifying those unlikely to respond to treatment, which included those with baseline azoospermia. Nonazoospermic patients with a baseline T-LH ratio <100 constituted the final fraction of the cohort. Under the assumption that all patients with nonazoospermia received a trial of anastrozole and all patients with azoospermia avoided treatment, this partitioning model was 98% sensitive and 33% specific for WHO-SCC upgrades. Notably, one patient in the cohort did not possess a baseline LH measurement and was excluded from partitioning analysis.

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portion of the cohort (n = 71/89, 80%) was further differentiated by those unlikely to respond to treatment. In the partitioned subset of those with azoospermia, 94% (n = 16/17) did not respond to a trial of anastrozole therapy. The remaining nonazoospermic patients with the T-LH ratio <100 (n = 54/71, 76%) exhibited a differential response to anastrozole, with 43% (n = 23/54) experiencing a clinically significant response to anastrozole. Under the assumption that all patients with nonazoospermia received a trial of anastrozole and all patients with azoospermia avoided treatment, this partitioning model was 98% sensitive and 33% specific for WHO-SCC upgrades (AUC: 0.77). Given these findings, men with nonazoospermia and a T-LH ratio ≥ 100 appear to be the ideal cohort for anastrozole therapy.

DISCUSSION

First approved in 1995 for locally advanced or metastatic breast cancer, anastrozole is commonly used off-label as empiric medical therapy to treat male infertility (2, 10). There are currently no society recommendations guiding clinicians regarding timing or patient selection for anastrozole therapy. Moreover, only limited data characterizing patient factors that predict a clinically significant response to this agent are currently available. Identifying infertile men likely to respond to anastrozole is important because doing so potentially expands noninvasive fertility treatment options and may minimize the need for costly assisted reproductive technology. Given that pregnancy is often a time-sensitive process, recognizing those unlikely to respond to anastrozole may permit earlier initiation of alternative treatment modalities.

In our multi-institutional cohort, 46% (n = 41/90) of patients initiated on anastrozole demonstrated a clinically significant response, with a median sixfold increase in sperm concentration (23.0 vs. 3.6 M/mL) among those who experienced a WHO-SCC upgrade. We observed that responders exhibited lower levels of pretreatment Gns with lower baseline LH (4.7 vs. 8.3 IU/L) and FSH (4.7 vs. 6.7 IU/mL). This finding is further reflected in the predictive power of the T-LH ratio, which was identified as the best differentiator of responder status on univariate screening with an AUC of 0.744. Enhanced predictive performance was seen also with baseline nonazoospermia (AUC: 0.627), a binary categorical variable defined by pretreatment cryptozoospermia or oligozoospermia, and semen concentration (AUC: 0.655) on univariate modeling. Collectively, these observations suggest patients are more likely to respond to anastrozole when they have lower pretreatment levels of Gns and at least some baseline sperm production, a finding demonstrated across our best-performing multivariable prediction models (Supplemental Table 3, available online).

Interestingly, the T-LH ratio was the best initial differentiator in our recursive partition analysis, and a ratio ≥100 was selected as the cutoff (Fig. 2). A T-LH ratio ≥100 commonly represents a patient with low pretreatment levels of LH and low-to-normal levels of T. The high rate of WHO-SCC upgrade among this subgroup is biologically sensible given that infertile men with inappropriately low Gn levels could benefit from peripheral E_2 reduction and decreased inhibition on the hypothalamic-pituitary-gonadal axis (17). Given the higher WHO-SCC upgrade frequency noted with increasing T and LH quartiles (Fig. 1B), this

hypothesis could be extrapolated to explain that patients with higher T-LH ratios represent a population with functional Sertoli and Leydig cells that could support enhanced spermatogenesis once properly stimulated. Future studies are needed to further validate this finding.

Although anastrozole has historically been prescribed for men with reduced T-E_2 ratios or excess adiposity (8, 9, 18) our study suggests this treatment may be considered for a more diverse group of patients. In this study, baseline E_2 concentration was treated as a binary categorical variable with a cutoff set at 25 pg/mL to reflect the higher detection threshold among various E_2 assays. We considered E_2 values <25 pg/mL to be null and reported the inverse ratio (E_2-T) to ensure real values could be analyzed. Interestingly, responder status was not associated with baseline E_2, E_2-T ratio, or BMI, and none of the variables reflective of pretreatment E_2 status were selected as a high-performing predictor of WHO-SCC upgrade during our univariate screen of baseline patient characteristics. Furthermore, a sensitivity analysis was performed to compare the performance of the E_2-T ratio (cutoff: $\geq$0.1) to the partition containing the T-LH ratio (cutoff: $\geq$100) and baseline nonazoospermia in distinguishing anastrozole responders from nonresponders (Supplemental Table 4, available online). Our new proposed partition model achieved greater sensitivity (98 vs. 54%), a higher negative predictive value (94 vs. 54%), and an equivalent positive predictive value (54 vs. 54%). This is not to say that anastrozole cannot benefit infertile men with elevated baseline E_2, but rather to highlight that the current study identified factors more predictive of a clinically significant response in sperm concentration in a larger number of patients.

We also note that our primary outcome, the WHO-SCC upgrade, represents a clinically meaningful endpoint that enables an expansion of infertility treatment options. For example, 31% (n = 20/64) of men with pretreatment TMSCs <5 M achieved posttreatment TMSC $\geq$5 M and became eligible for intrauterine insemination (19). The addition of intrauterine insemination as a treatment option potentially avoids an expensive and potentially protracted trial of in vitro fertilization. Additionally, a remarkable 29% (n = 26/90) of the cohort achieved posttreatment normozoospermia: 25% of those with severe oligozoospermia (n=8/32) and 57% of those with oligozoospermia (n = 16/28) at baseline achieved sperm concentrations of >15 M/mL after initiation of anastrozole, an upgrade that potentially permits a trial of natural conception.

Initiation of anastrozole is not without risk. Before starting therapy, men must be counseled on potential side effects, including reduced bone mineral density, headaches, nausea or diarrhea, and mood changes (20–22). Anastrozole administration was also associated with a WHO-SCC downgrade in 12% (n = 11/90) of the cohort, including 2 men with cryptozoospermia who developed posttreatment azoospermia. Given the lack of multiple posttreatment semen analyses, it is possible that these downgrades represent natural variations in sperm production, but this cannot be determined from our data. Future prospective work evaluating anastrozole among infertile men should include multiple posttreatment semen analyses to evaluate potential variability in sperm production. Despite these drawbacks, anastrozole should be considered by a wide array of patients given the potential expansion of infertility treatment options available with enhanced sperm production.

Special attention should be paid to those with baseline azoospermia. In our cohort, 84% (n = 16/19) of patients with pretreatment azoospermia did not respond to anastrozole. Similarly, a cohort study by Shoshany et al. (11) found that all men with baseline azoospermia (n = 28) remained azoospermic after a 4-month course of anastrozole. Our data demonstrate an unfavorable outcome with this approach and provide an impetus for patients with azoospermia with time-sensitive fertility goals to consider alternative therapies. Whether anastrozole treatment improves outcomes with microdissection testicular sperm extraction outside of the Klinefelter population, where limited data support its use, remains to be studied (23).

This cohort study has limitations that are important to consider. Notably, the retrospective nature of this research prevents the definitive assertion of causality between treatment and response. Laboratory studies were completed at the institution where patients sought care, which introduces the potential for measurement bias across the hormone and semen parameters analyzed. Changes in semen and hormone parameters were affected potentially by test-retest variability. Semen parameter measurements were taken from a single measurement before and after treatment, making us unable to comment on the duration of benefit for responders. We were unable also to calculate the mean duration of therapy because treatment stop dates were not always recorded explicitly. Variation in anastrozole dosing was a potential confounder, but cumulative weekly exposure was not associated with responder status or a change in sperm concentration. Utilizing the WHO-SCC upgrade as the primary end point could be criticized also given that it is an indirect marker of male fertility, unlike pregnancy and live birth rates (data that were unavailable). Despite this limitation, the WHO-SCC upgrade reflects clinically significant changes in sperm concentration and would enable access to expanded assisted reproductive technology options. Additionally, the cohort demonstrated heterogeneity between patients treated at CCF and UCLA cohorts, especially regarding age, BMI, anastrozole dose, and varicocele history (Supplemental Table 5). However, when the institution was input as a predictor variable in our multivariable analyses, its inclusion had a nonsignificant effect on model performance. We believe that this heterogeneity broadens the generalizability of our findings given that predictor variables were identified among a diverse patient cohort.

Despite these caveats, to our knowledge, this study represents the first multi-institutional investigation of a previously understudied population and is the largest study to date to examine anastrozole treatment among infertile men with altered semen and clinical parameters. Additional research is required to validate the predictor variables identified in this study, ideally through a large, diverse, prospective cohort study. Further investigation is needed also to assess the therapeutic effect of concomitantly prescribing anastrozole with

other agents (e.g., CC or hCG) and to identify whether alternative AIs (e.g., letrozole and testolactone) produce similar effects on semen and hormone parameters.

CONCLUSION

Initiation of anastrozole was associated with decreased serum E_2 levels, increased serum Gn levels, and clinically improved semen parameters in roughly half of infertile men. Baseline sperm production and higher T-LH ratios were predictive of a WHO-SCC category upgrade. E_2 levels, the E_2-T ratio, and BMI demonstrated poor predictive power in this cohort. Men with pretreatment azoospermia respond poorly to anastrozole and should consider other treatment modalities. Non-azoospermic infertile men with a T-LH ratio ≥ 100 (and to a lesser extent those with a ratio <100) may benefit from treatment with anastrozole and counseled on the anticipated probability of a clinically significant improvement in semen parameters. Doing so may expand the therapies available to couples facing male factor infertility and permit a trial of less invasive or less expensive fertility treatments.

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La testosterona y la hormona luteinizante predicen la mejoría en los parámetros seminales en hombres infértiles tratados con anastrazol

Objetivo: Identificar los factores de los pacientes asociados con una mejoría clínicamente significativa en los parámetros del semen entre hombres infértiles tratados con el inhibidor de la aromatasa anastrazol.

Diseño: Estudio de cohorte retrospectivo multi institucional.

Lugar: Dos centros médicos académicos terciarios.

Paciente(s): Un total de 90 hombres infértiles tratados en 2 centros médicos académicos terciarios quienes cumplieron con los criterios de inclusión y obtuvieron análisis de semen previo y post tratamiento.

Intervención(es): Prescripción de anastrazol (mediana 3 mg/semana).

Medidas principales de resultado: Aumento en la categoría de concentración de semen de la Organización Mundial de la Salud (OMS-SCC). Se realizaron análisis de regresión logística univariada, regresión logística multivariable y partición para identificar factores estadísticamente significativos de los pacientes capaces de predecir la respuesta al tratamiento.

Resultado(s): Con el tratamiento con anastrazol, el 46 % (n = 41/90) de los hombres respondieron favorablemente con un aumento en OMS-SCC y el 12 % (n = 11/90) experimentó una disminución. Los que respondieron exhibieron niveles previos al tratamiento más bajos de hormona luteinizante (LH, 4,7 versus 8,3 UI/L) y hormona folículo estimulante (4,7 versus 6,7 UI/mL), niveles más altos de testosterona previo al tratamiento (T, 356 versus 265 ng/dL) y nivel basal similar de estradiol (E2, 73% vs. 70% con nivel detectable). Los parámetros basales del semen difirieron, los que respondieron al anastrazol demostraron mayor concentración inicial de semen (3,6 frente a 0,3 M/ml) y mayores recuentos totales de espermatozoides móviles (3,7 frente a 0,1 M). La terapia con anastrazol convirtió el 29% (n = 26/90) de la cohorte a normozoospermia y permitió el acceso a la inseminación intrauterina en el 31% (n = 20/64) de pacientes previamente no elegibles. De manera interesante, ni el índice de masa corporal ni el nivel inicial de E2 o la tasa E2-T se asociaron con cambios en OMS-SCC. La regresión logística multivariable reveló que la relación T-LH (odds ratio: 1,02, intervalo de confianza del 95 %: 1,00–1,03) y la no azoospermia inicial (odds ratio: 9,4; intervalo de confianza del 95%: 1,1–78,9) son predictores estadísticamente significativos de la mejora del WHOSCC (área bajo la curva característica operativa del receptor: 0,77). El modelo de partición final fácil de usar que consta de la relación T-LH R100 y la no azoospermia inicial fueron 98 % sensibles y 33 % específicas para las mejoras de WHO-SCC (área bajo la curva:0,77).

Conclusión(es): La terapia con anastrazol disminuye los niveles séricos de E2, aumenta las gonadotropinas séricas y mejora clínicamente los parámetros seminales en la mitad de los hombres con infertilidad idiopática. Los hombres infértiles no azoospérmicos con ratios T-LH R100 probablemente se beneficien del tratamiento con anastrazol independientemente del nivel inicial de E2 o la relación E2-T. Los hombres con azoospermia rara vez responden al anastrazol y se les debe aconsejar tratamientos alternativos.