Defining hypogonadism in male partners of couples with unexplained infertility

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Introduction: *Men with unexplained infertility (UI) should undergo an initial hormonal evaluation including serum FSH and total testosterone (TT). Unfortunately, there is no consensus regarding which TT cut point should be used to define hypogonadism in such men. To determine the best definition for hypogonadism, three different, literature-based TT cut points were used to assess associations between TT and semen parameters. The hypothesis was that the lowest TT cut point would associate with poorest sperm parameters.*

Materials and methods: *We performed an IRB-approved retrospective chart review of 247 consecutive males presenting for evaluation of male factor infertility. After exclusions, basic statistics and correlation analysis of semen analysis parameters, TT, age, and body mass index (BMI) were evaluated on 128 men (age 34+/-33.5) categorized by three different TT cut points: 65 males were hypogonadal according to a TT cutoff of < 264; 16 with a cutoff of 264-300; 44 with a cutoff of 301-400; and 42 with a TT over 400 ng/dL. Basic statistics, one-way ANOVA and Levene comparative analysis were performed. Besides a negative correlation between TT and BMI, there was no significant association between the three TT literature-based cut points and the other studied parameters. These findings were further supported by multiple comparison analyses.*

Results: *For men with UI, regardless of how hypogonadism was defined, no relationship between semen parameters and TT was found.*

Conclusion: *Conventional, TT-based definitions of male hypogonadism in the setting of UI need to be clarified. Clinically relevant, accurate and reproducible multivariable biomarkers need to be investigated to further advance best practices for treating men with UI.*

Key Words: male infertility, unexplained infertility, hypogonadism, semen analysis, pregnancy

Introduction

In about 50% of cases, a male factor for infertility exists, with 25%-30%[1-3] of these cases categorized as UI. Such a diagnosis is frustrating for both the infertile couple and the provider, as the medical etiology is unknown—thereby lacking a clear treatment guideline. Some experts believe that all infertile males should have an endocrine evaluation which includes, at a minimum, a serum FSH and testosterone (T). Alternatively, the American Urological Association (AUA) best practice statement reports that there is no consensus on this recommendation.[4] Since Sertoli cells thrive in an environment of extraordinarily high T, low T levels may result in defective sperm production.[5] Low T scenarios become more prevalent as men exceed 40 years of age[6] and/or stress their bodies with either chronic disease or increasing body mass index (BMI).[7] As such, it is well established that being overweight or obese is associated with significant reductions in total and free testosterone.[8]

Urologists have observed that male unexplained infertility (UI) is often noted in men who have both an abnormal semen analysis and low total testosterone (TT). As such, serum TT levels are frequently used to guide treatment options for such men. Specifically, men without evidence for primary testicular failure (having normal LH/FSH) who have low TT levels are frequently offered empiric medical therapies (EMT). Such management

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strategies strive to upregulate LH and FSH production, which in turn, promotes both spermatogenesis and enhanced intratesticular testosterone (ITT).

In spite of a steep 100-fold[9-11] testicular-to-serum T gradient, it is unclear if serum TT is predictive of ITT levels or Sertoli cell function. Although testing in some animal models has shown that a minimal T level is required for spermatogenesis,[12] the minimal level for human spermatogenesis is not well defined.[13] Moreover, there is no clear definition for low TT (hypogonadism) below which, empirical treatment for UI should be considered. Currently, the most common cut point appears to be 300 ng/dL[14,15] with other cut points often used in practice.

To the best of our knowledge, this is the first study looking at a cohort of men with UI, attempting to clarify which definition of low-TT had the greatest negative effect on semen parameters. Our hypothesis was that the lowest TT cut point would associate with poorest sperm parameters. We chose three cut points to define low TT: 264, 300, and 400 ng/dL. The 300 ng/dL point represents the AUA guideline[1] definition for low TT and is used often in the male infertility literature.[14,15] Secondly, a contemporary cut point was suggested by Travison[16] who looked at 4 cohorts of healthy young men between the ages of 19 and 39 and determined that their TT ranged from the 2.5th percentiles of 264 ng/dL and 916 ng/dL (using the harmonized normal range in a healthy non-obese population of European and American men). Third, the endocrine society uses 400 ng/dL to define low T—above which, replacing symptomatic men with testosterone will unlikely change their andropause symptoms.[17]

Material and methods

Study sample.

We performed an IRB-approved retrospective chart review of consecutive males presenting to an infertility clinic for evaluation of male factor infertility over a 24-month period. After excluding those having other than UI, data of 247 consecutive males (35.0 +/- 6.21 years) were analyzed.

Statistical analysis

Basic statistics, Spearman and Kendal tau correlation analyses tested for significance between mean values by comparing variances (one-way ANOVA, Welsh multiple means test), Levene test of homogeneity of variances and multiple comparison analyses (Dunnett test) were performed. IBM-SPSS statistics-25 and Statistica-7 software were used. Significant cutoff was defined by p value ≤ 0.05.

Results

After exclusions, basic statistics and correlation analysis of volume, concentration, sperm parameters (motility and strict morphology) and TT level were evaluated on 168 men (age 34+/-33.5): 65 males who were hypogonadal according to a TT cutoff values of < 264 (group 1, age 34.1+/-5.66); 16 with cutoff of 264-300 (group 2, age 34.88+/-6.28); 44 with a cutoff of 301-400 (group 3, age 35.1+/-5.91); and 42 with a TT over 400 ng/dL (group 4, age 32.7+/-5.1).

Table 1 shows a descriptive statistics summary. The 95% confidence interval comparisons show no significance for any variable other than BMI. Also, we did not find significant differences between any paired neighbor groups nor any ordered groups when they were combined. We observed a highly confident negative correlation between descending ranked order TT groups (from one to four) and BMI (Spearman corr = -0.50, $p < 0.0001$) in the entire patient group.

Breakdown TT categories and one-way ANOVA analysis

We computed a one-way Analysis of Variance (ANOVA) (IBM-SPSS statistics, version 25) for the 4 TT categories defined by cutoff values 264, 300, 400. First, we calculated descriptive statistics and correlations for dependent variables in each of the groups defined by one or more grouping (independent) variables (categorical predictors).

One-way ANOVA comparative analyses of the mean values of volume, concentration and two sperm parameters given by TT groups were performed on 74 men: 26 males who were hypogonadal according to a TT cutoff of < 264 (group 1); 9 with cutoff of 264-300 (group 2); 22 with a cutoff of 301-400 (group 3); and 17 with a TT over 400 ng/dL (group 4), Table 2. The test did not show any significant trends with these variables in testing the association with TT ordered grouping, Figure 1A-D. The one-way ANOVA test demonstrated that for each studied variable null hypothesis (no influence of TT level) is accepted (due to $p > 0.1$), excluding BMI, which was high significant ($p < 0.01$). Welch's Test in IBM-SPSS-25 ANOVA module is an alternative robust parametric test of equal population means when equal population variances in groups is not assumed. Results of this test confirmed the results of one-way ANOVA test, Table 2.

Additionally, we used Levene's test which demonstrated similar variance estimations across the 4 TT groups, suggesting validity of assumption of one-way ANOVA test (results are not shown).

TABLE 1. **Patient characteristics of male partners of couples with unexplained infertility. Descriptive statistics**

TT category group*	Valid n	Median	Mean	SD	CI-0.95	CI 0.95
Group 1 (TT < 264)						
Semen volume (mL)	57	2.1	2.5	1.69	2.0	2.9
Semen concentration (10^6/mL)	54	1.7	17.5	32.91	8.5	26.5
Semen motility (%)	44	35.3	36.6	27.90	28.1	45.1
Strict morphology (%)	27	2.0	5.0	6.39	2.4	7.5
TT (ng/dL)	65	199.0	185.8	58.24	171.4	200.3
Age (years)	65	34.0	34.1	5.66	32.7	35.5
Body mass index (kg/m^2)	64	34.5	36.2	10.27	33.6	38.7
Group 2 (TT 264-300)						
Semen volume (mL)	13	2.0	2.2	1.28	1.5	3.0
Semen concentration (10^6/mL)	14	15.7	24.3	39.70	1.3	47.2
Semen motility (%)	11	31.5	31.7	27.37	13.3	50.1
Strict morphology (%)	9	5.0	10.6	16.98	-2.5	23.6
TT (ng/dL)	16	279.5	279.9	11.49	273.8	286.0
Age (years)	16	33.5	34.9	6.28	31.5	38.2
Body mass index (kg/m^2)	15	30.4	31.4	7.36	27.4	35.5
Group 3 (TT 301-400)						
Semen volume (mL)	39	2.5	2.6	1.84	2.0	3.2
Semen concentration (10^6/mL)	38	5.1	21.7	43.88	7.3	36.2
Semen motility (%)	34	30.0	32.0	23.46	23.8	40.2
Strict morphology (%)	24	2.0	8.1	18.16	0.5	15.8
TT (ng/dL)	44	345.0	346.3	28.23	337.7	354.9
Age (years)	44	34.0	35.1	5.91	33.3	36.9
Body mass index (kg/m^2)	43	28.5	29.5	6.61	27.5	31.6
Group 4 (TT > 400, > 1600 ng/dL were excluded)						
Semen volume (mL)	36	2.2	2.5	1.59	2.0	3.0
Semen concentration (10^6/mL)	35	2.3	19.3	36.34	6.8	31.8
Semen motility (%)	31	43.0	33.3	22.46	25.1	41.5
Strict morphology (%)	18	2.0	5.2	6.48	1.9	8.4
TT (ng/dL)	42	488.0	530.5	139.85	486.9	574.1
Age (years)	43	32.0	32.7	5.11	31.1	34.3
Body mass index (kg/m^2)	43	25.7	26.7	4.28	25.4	28.1
All 168 patients						
Semen volume (mL)	145	2.3	2.5	1.66	2.2	2.8
Semen concentration (10^6/mL)	141	3.7	19.8	37.30	13.5	26.0
Semen motility (%)	120	34.0	34.0	25.06	29.4	38.5
Semen morphology (%)	78	2.5	6.6	12.47	3.8	9.4
TT (ng/dL)	168	305.5	331.4	185.70	303.1	359.7
Age (years)	168	33.5	34.1	5.67	33.2	34.9
Body mass index (kg/m^2)	165	29.5	31.6	8.75	30.2	32.9
Group 2 + Group 3						
Semen volume (mL)	52	2.5	2.5	1.71	2.0	3.0
Semen concentration (10^6/mL)	52	7.8	22.4	42.43	10.6	34.2
Semen motility (%)	45	31.5	31.9	24.15	24.7	39.2
Strict morphology (%)	33	3.0	8.8	17.62	2.5	15.0
TT (ng/dL)	60	328.0	328.6	38.63	318.6	338.6
Age (years)	60	34.0	35.0	5.95	33.5	36.6
Body mass index (kg/m^2)	58	28.7	30.0	6.80	28.2	31.8

*in normal: semen volume ≥ 1.5 mL, sperm concentration ≥ 15 10^6/mL, total sperm motility ≥ 40%, strict morphology ≥ 4%.

TABLE 2. **ANOVA and equality of means tests**

ANOVA						
Variable	**S. volume**	**S. motility**	**S. concentration**	**Strict morphology**	**Age**	**BMI**
F-test	0.853	1.442	0.024	1.041	0.939	8.202
p value	0.47	0.238	0.995	0.38	0.427	< 0.0001
Equality of means						
Welch F-test	0.994	1.21	0.02	0.809	0.793	7.872
p value	0.409	0.324	0.996	0.501	0.508	0.001

We used post-hoc multiple comparison Dunnett test determining for a given variable which TT group means are different. This test demonstrated differences only between paired TT groups and the BMI data. These results were supported by the Kendal tau correlation analysis, which counts a non-parametric rank order association-based measure and therefore is invariant to any cutoff numbers and the cutoff values, Figure 2A-F. We found that only in the TT level - BMI pair, the Kendal tau correlation coefficient was significant (r= -0.347, p < 0.0001, Figure 2F). We also found weak correlation between BMI and semen volume (r =-0.141, p < 0.05).

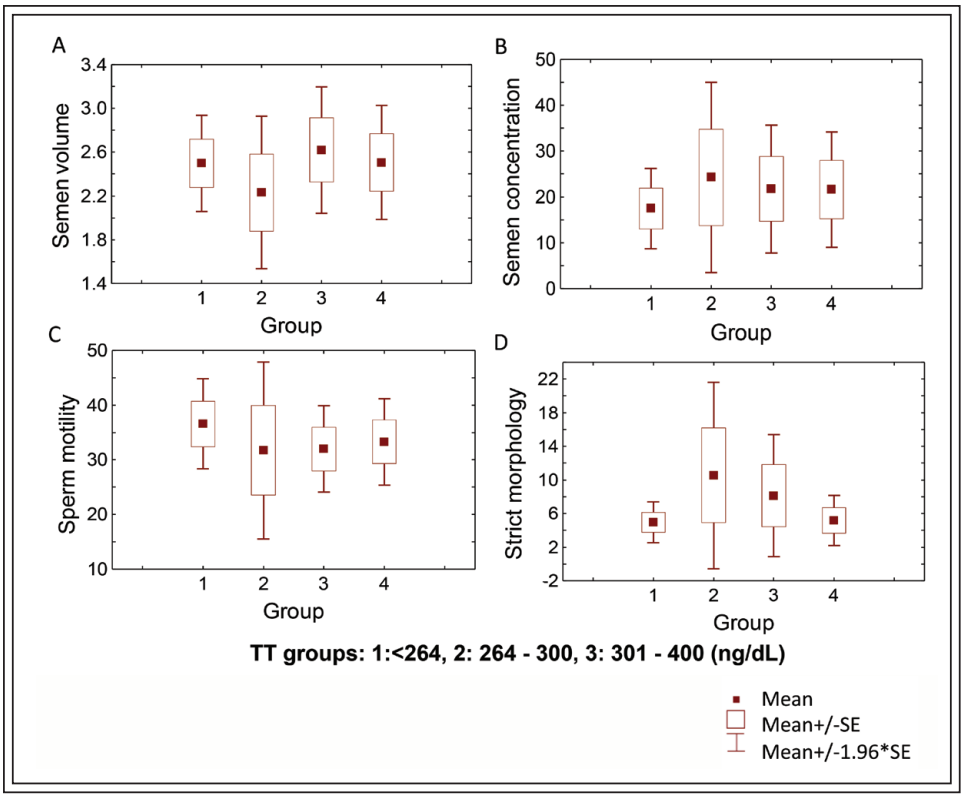

Figure 1. One-way ANOVA test associated TT categories (box and whisker plots). **A:** semen volume, **B:** semen concentration, **C:** sperm motility, D: strict morphology. TT (ng/dL): Group 1 < 264, Group 2 = 264-300, Group 3 = 301-400, Group 4 > 400 (ng/dL).

Discussion

Lacking reliable low-invasive predictive biomarker(s) and treatment guideline consensus for men with UI often requires EMT and/or assisted reproductive techniques. EMTs, such as selective estrogen receptor modulators (SERMS), are often used in hypogonadal men as this treatment option is non-invasive, has minimal side-effects, and is generally considered effective in improving semen parameters.[18] Whether or not the improved semen parameters translate into a higher pregnancy rate is unclear: (1) some reviews demonstrated non-significant improvement[19,20] while, on the other hand, (2) a meta-analysis of 11 randomized controlled trials found that SERM therapy was associated with a statistically significant increase in the pregnancy rate (OR, 2.42;95% CI, 1.47-3.94;

p = 0.004) sperm concentration(p = 0.001), and sperm motility (p = 0.03). One potential problem with such studies is not having a clearly defined TT cut point to use for determining which man with both UI and hypogonadism, would optimally respond to EMT.

According to our results, for men with UI, there is no adjusted TT cut point below which semen parameters declined significantly. This held true for the three different hypogonadism definitions chosen for this study, Figures 1, 2, Tables 1, 2). The TT cutoff value concept is rejected by our analyses using both discrete and continuum data points. These results suggest that the determination of optimal spermatogenesis and fecundity may require more than a single TT cut point. As such, the detection of other biological variables need to be considered as a compliment to TT. Such alternate variables need to be identified to enhance the sensitivity specificity, accuracy and reproducibility of which men with idiopathic infertility symptoms would most benefit from EMT's. Likely, an algorithm including other clinical variables (such as insulin, glucose, BMI and age) in combination with new high informative accurate and reproducible biomarkers (DNA, RNA, proteins, extracellular vesicles) will be necessary. Until then, identifying men with low TT remains an important part of working up couples where the male partner has UI as prior work has demonstrated that low TT (< 264 ng/dL) was associated with a 40 percent drop in live birth rate in addition to a greater risk for teratospermia.[21]

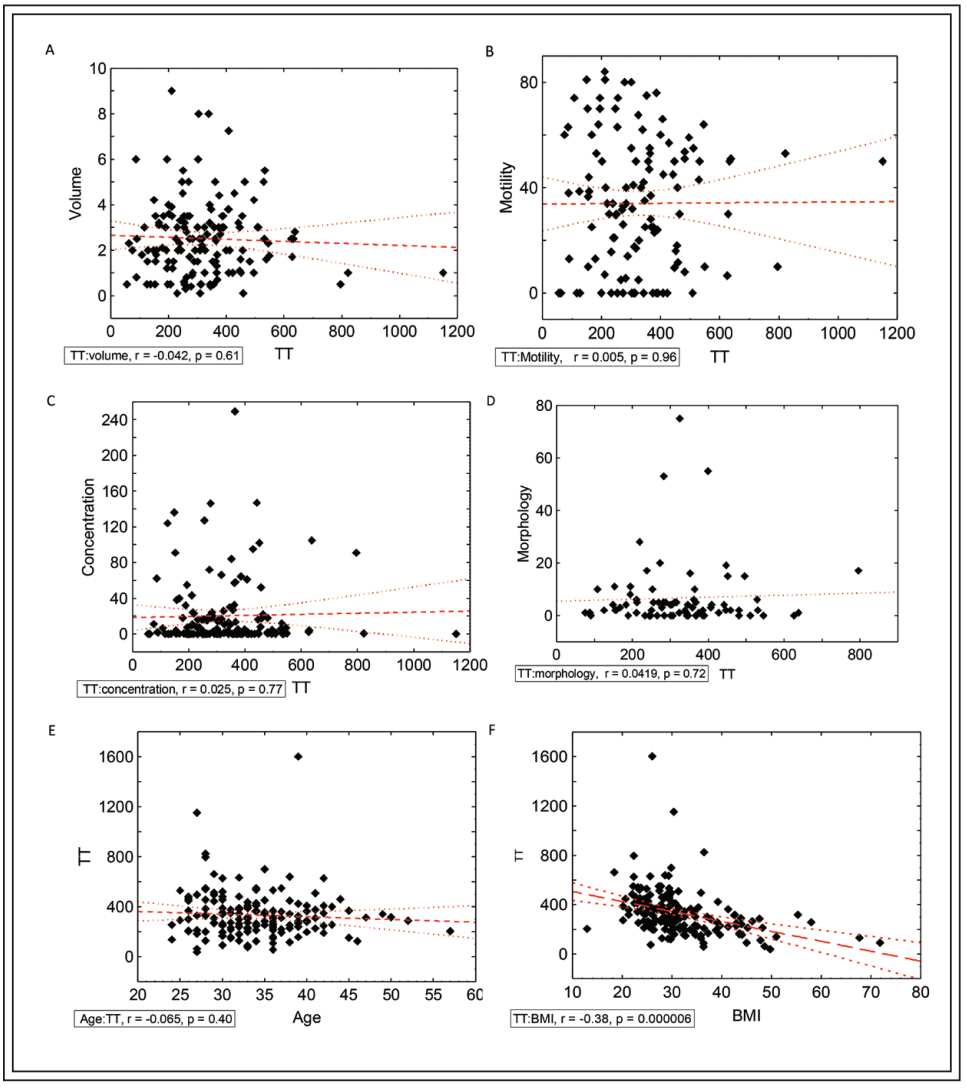

Figure 2. Bi-variate distributions of TT vs. other variables and correlation analysis **(A-F)**. Regression lines with 95%- confidence interval are shown. TT cutoff value 264 ng/dL (direct line) is shown on panels **A-D**. Significant regression is observed between TT and BMI only **(F)**.

This study does have limitations. Foremost, this is a retrospective analysis of men with idiopathic infertility. There was no strict control for the time of day for the TT blood draw. As such, this may have affected TT measurements, as males with later blood draws may be falsely identified as having low TT due to the diurnal secretion of testosterone production in men. However, due to common practice morning detection and relatively large number of the cases studied this factor may be not the major source of biological variability. Secondly, only one baseline TT was collected, and thirdly, symptoms of hypogonadism were not assessed.

Thirdly, TT measurements on more than one day, as well as tests for levels of hormones related to testosterone that could improve diagnostic accuracy. Lastly, there

was no free testosterone or calculated bioavailable testosterone available for review. These conditions may affect accuracy and reproducibility of TT level testing.

The current guidelines are limited by the inability to generalize recommendations to a heterogeneous patient sample. As the field of infertility continues to expand, the utility of guidelines combined with physician clinical judgement will remain prominent in the treatment of male-factor infertility.[22]

As the rate of growth of medical knowledge continues to increase, the role for and utility of expert guidelines will become increasingly important. Future guidelines will likely reflect novel treatment modalities and will continue to provide a basic framework upon which physicians may base clinical judgements.[22]

Future guidelines will continue to incorporate new informative diagnostic biomarkers and discoveries for treatment in the management of infertility. Numerous investigations are ongoing into determining predictive associations between semen findings and overall sperm quality beyond the currently accepted WHO semen characteristics.[22]

Conclusions

These data call into question the meaning of conventional, TT-based definitions of male hypogonadism in the setting of UI. Diagnosing and confirming low testosterone requires multi-variable design, thorough lab testing and careful interpretation of the results. Complementary and new and reproducible variables (biomarkers) will need to be identified and investigated to further clarify the best practice for treating men with UI. Our study reiterates an urgent need to uncover the causes and mechanisms behind, and potential treatments of, male hypogonadism in couples with UI. □

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