International Scholars Journals

International Journal of Urology and Nephrology ISSN: 2756-3855 Vol. 10 (9), pp. 001-021, September, 2022. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

How and When to Evaluate Testosterone Deficiency: Controversies and Consensus Among Specialties Worldwide

AUTHORS: Sara Q. Perkins[1], David Fumo[2] MD, Puneet Sindhwani[2] MD.

ABSTRACT

The worldwide prevalence of testosterone deficiency (TD) is increasing due to an aging population. However there is discrepancy surrounding its diagnosis, both between countries and major specialty societies. The purpose of this review is to compare and contrastcurrent guidelines across the world to assess variability in the diagnosis and evaluation of TD. There were no available guidelines from Asian, African or South American specialty societies in English language literature. Guidelines from Canada, Europe, and the United States, including the American Association of Clinical Endocrinologists (AACE) the American Urologic Association (AUA), and the Endocrine Society are evaluated. A literature search was performed using Pubmed, Uroweb, the AUA and AACE websites to evaluate the most recent guidelines on hypogonadism. Guidelines and the level of

evidence supporting these are compiled in Tables. All guidelines concurred that testosterone replacement therapy (TRT) is indicated in patients with symptoms of hypogonadism in combination with biochemically low testosterone but there was no consensus with respect to the cut off -value for low testosterone,diagnostic methodology, preferred assays or screening at risk populations. This highlights the differences in world health care delivery in the evaluation and treatment of TD and need for further research to build a consensus usingbest available evidence. This article provides the most updated and concise review of the controversies and consensus in the diagnosis and evaluation of TD.

[1] University of Toledo College of Medicine and Life Science

[2]Department of Urology, University of Toledo College of Medicine and Life Sciences

INTRODUCTION

Testosterone Deficiency (TD) or hypogonadism in adult men is both a clinical and biochemical diagnosis[1, 2]. TD is defined as the presence of clinical signs and symptoms as well as documented serum testosterone levels below accepted laboratory ranges[2, 3]. Ultimately, low testosterone may affect multiple organ systems resulting in both physical and psychosocial consequences[2, 3]. The causes of TD range widely making diagnosis and management difficult for practitioners[4]. Typically testosterone replacement therapy (TRT) is recommended treatment for TD[5].

Several professional bodies have published guidelines on the subject of TD and TRT [1, 3]. As the world's population ages TD prevalence is expected to increase making safe and effective diagnosis and management more important[6, 7]. The purpose of this review is to evaluate the variability in diagnosis and evaluation of TD in published guidelines available in English literature. Guidelines from Canada[8], Europe[9], and the United States including the American Association of Clinical Endocrinologist (AACE) [10] the American Urologic Association(AUA)[11-14], and the Endocrine Society[15] are included in this review.This review will highlight consensus opinions regarding best practices for the diagnoses and management of TD. It will also identify discrepancies between guidelines from each association and between the two most recent TD guidelines from the AUA.

METHODS

A literature search was performed to evaluate the most recent guidelines on hypogonadism/TD. The search for the available guidelines in published English literature was performed October 22nd, 2017 via Pubmed, Uroweb, AUA, and AACE websites. In addition, new guidelines by the AUA and the Endocrine Society were released and included for review on April 2nd, 2018. Guidelines were extracted from the following four organizations:Canadian Men's Health Foundation Multidisciplinary Guidelines Task Force on Testosterone Deficiency (CMHF), European Association of Urology (EAU), and AUA, American, AACE, and the Endocrine Society. The literature was reviewed with an emphasis on the organizations' guidelines, as well as position statements, white papers, and consensus statements. Emphasis was placed on comparing when to evaluate for TD, laboratory testing, standardization,and adjunctive testing.

GUIDELINES

Description and comparison of levels of evidence and grade of recommendations can be found in Appendix 1 and Appendix 2.

EAU

The EAUMale Hypogonadism Panel worked to create the current recommendations. Recommendations by the panel were based on a systematic review of literature that includedarticles published before November 2014, the current recommendations were constructed using 118 citations.The articles with the highest level of evidence were selected in accordance theOxford Centre for Evidence-Based Medicine Levels of Evidence[9].

CMHF

The CMHF Multidisciplinary Guidelines Task Force on TD commissioned 2 systematic performed by a librarian and a pharmacist in December 2013 and updated in April 2014.The final guidelines were based off of 454 citations. The Task Force adopted consistent language to describe the level of evidence and strength of recommendations, as recommended by the GRADE Working Group[8, 16].

“OLD” AUA

The documents referred to as the “old” AUA guidelines throughout the review encompass now archived documents including: AUA Position Statement on Testosterone Therapy most recently revised in 2015[13], AUA White Paper on Laboratory Diagnosis of TD 2013[11], and AUA consensus statement on Testosterone Testing published in 2010[12]. The aforementioned documents were the only documents available on the AUA website when performing a search for testosterone deficiency guidelines in October of 2017.

2018 AUA

The AUA appointed a panel to construct the newest guidelines published in 2018. The authors utilized a systematic review that encompassed articles published between January 1, 1980- February 6, 2017, 546 references were used to support guidelines statements. Levels of evidence and strength of recommendations utilized AUA nomenclature, linking statement type to evidence of strength[14].

AACE

The AACE Hypogonadism Task Force constructed the guidelines for

clinical practice for adult men with hypogonadism published in 2002. Guidelines were constructed based off of literature reviews, with the use of 77 citations. Grades and level of evidence were not presented in the guidelines[10].

ENDOCRINE SOCIETY

The Endocrine Society guidelines were based on the best available evidence found in two systematic reviews, other reviews, and individual studies. The guidelines have 156 citations total and were developed using the Grading of Recommendations,Assessment, Development, and Evaluation approach to describe the strength of recommendations and the quality of evidence. The guidelines published in 2018 were to serve as an update to the “Testosterone Therapy in Men With Androgen Deficiency Syndromes”published in 2010[15]. Based on the above evaluation of sources the 2018 AUA guidelines has the most robust literature review with a total of 456 sources. While only 77 articles were used in the formulation of the AACE guidelines.

DISCUSSION

When to Evaluate for TD

Classic signs, symptoms, and general recommendations on when to diagnostically evaluate a patient for TD are similar across all guidelines.Recommendations on when to diagnostically evaluate a patient, as well as unique populations that each society recommends for testing is presented in Table 1.All societies agree that diagnostic evaluation of TD is to be pursued when both clinical and historical findings suggestive of TD are present[8-11, 14, 15].However, guidelines varied in regards to special patient populations that

could be considered for diagnostic evaluation of TD. European guidelines present the highest number of unique populations that can be considered for evaluation of TD while the Endocrine Society and AACE present the least. Unique populations eligible for evaluation of TD by the EAU include history of radiated pituitary mass and moderate to severe COPD[9]. In contrast, 2018 AUA guidelines recommendconsideration for patients that have undergone testicular radiation or chemotherapy exposure[14]. The CMHF is the only group to recommended consideration of TD evaluation in men with erectile function that have failed PDE-5 inhibitor therapy[8, 16].

EAU,CMHF, 2018 AUA, and Endocrine Society*do not*recommend screening for TD in patients that are symptom free (EAU: C;3, CMHF: Strong recommendation; Moderate-quality evidence, Endocrine Society: 1, ++)[8, 9, 15].TD questionnaire use is discouraged by the EAU, CMHF, and 2018 AUA due to their low specificity(EAU: n/a, CMHF: Strong recommendation; Moderate-quality evidence, AUA: Conditional recommendation; Grade C)[8, 9, 14].

In regards to formal diagnosis the EAU suggests that diagnosis be restricted to only men with persistent symptoms(C; 3)[9]. Both AUA guidelines agree and add diagnosis should be made when patients have low total testosterone (Old AUA: n/a, 2018 AUA: Moderate recommendation; Grade B)[14]. The Endocrine Society requires lab confirmation of unequivocally and consistently low total serum testosterone and/or free testosterone on two separate occasions.(1, +++)[15].CMHFmakes a weak recommendation that in

the presence of a “convincing” clinical picture in combination with uncertain labs a trial of TRT is an acceptable approach to definitively diagnose TD [8].

Laboratory values, collection, and analysis

Table 2 illustrates a comparison of lab values, lab collection, and second line testing thatare recommended.The most aggressive lower limit of testosterone was by the AACE (below 200 ng/dl). While morning collection was agreed on by all guidelines specificity of morning differed between guidelines. Preferred method of analysis differed between guidelines with most agreeing immunoassay, mass spectrometry, or liquid chromatography be utilized. The AACE was the only guidelines to recommend equilibrium dialysis and free T index. All guidelines, except the 2018 AUA, recommended SHBG and free T measurement as second line confirmatory testing.

Low Testosterone Definition

The AUA conducted an evaluationof randomized control trials (RCTs) and found the median baseline total testosterone of 249 ng/dL(IQR 233-283)supported the cut-off value recommendation (Moderate recommendation; Grade B) [17-21]. In contrast, the old AUA noted that use of a rigid total testosterone of less than 300 ng/dL could lead to under treatment of men with significant symptoms or unnecessary treatment in asymptomatic patients. Similarly, the EAU compiled data from three large community based samples to make the recommendation that values below 230.1nmol/L-348.7nmol/L for total testosterone and 18.8ng/dL for free testosterone can be used to diagnose TD (A; 1)[9, 22-24]. The Endocrine Societydefines the lower limit of normal of total

testosterone using the Center for Disease Control (CDC) standard of 264 ng/dL[15]. This lower limit should be utilized when a CDC verified assay is the method of analysis, but in labs not certified by the CDC they cite considerable variation in reference range depending on the assay and population[25]. The Canadian guidelines did not define low testosterone explicitly, however, they recommend testosterone levels be measured with assays certified by the CDC standardization program (Strong recommendation, High quality evidence)[8]. The most aggressive lower limit of normal was set by the AACE as they recommend that men with symptoms and a total testosterone of less than 200 ng/dL could be potential candidates for testosterone therapy[10].

Laboratory collection

All societies have the general consensus that testosterone collection should take place in the morning and in the fasting state (EAU: A; 2, CMHF:Strong recommendation; Moderate evidence, AACE: n/a, Old AUA: n/a, 2018 AUA: Strong recommendation; Grade A, Endocrine Society: 1; +++) [8-10, 14, 15, 26]. The AUA defined “morning” as within 3 hours of awakening, to accommodate night shift schedules, other guidelines did not specify this [14]. Only old AUA, 2018 AUA, and the Endocrine Society guidelines recommend the delay of collection whenpatientsare acutely ill(older AUA: n/a, 2018 AUA: Strong recommendation; Grade A, Endocrine Society: 1; +++)[1, 11, 14, 15, 27].

Analysis tools and standardization

Accepted tools to analyze testosterone levels differ internationally. The EAU suggests that testosterone levels should be measured utilizing a “reliable”

method (A; 1) [9]. They cite that both immunoassay and mass spectrometry as viable options that produce valid results[9]. Similarly the old AUA, 2018 AUA, and Endocrine Society guidelines support the use of immunoassays(old AUA: n/a, 2018 AUA: n/a Endocrine Society: 1; +++). The 2018 AUA addsthat while immunoassay is an acceptable toolto analyze testosterone that liquid chromatography and mass spectrometry should be preferentially utilized where possible to limit variability and maximize accuracy. Liquid chromatography with mass spectrometry offers more specificity and sensitivity with higher precision especially in the lower range of total testosterone concentrations compared to immunoassays[11, 14, 15]. The old AUA and the 2018 guidelines note that formal recommendations regarding method of analysis cannot be made due to differing accommodations and availability at individual facilities[11, 14].

In contrast, AACE suggests the use of equilibrium dialysis and free testosterone index to determine serum testosterone levels[10]. The AACE explicitly recommends *against* the measurement of free testosterone with analog displacement assay citing its unreliability[28-31]. The CMHF guidelines do not cite specific tests in their guidelines.

Accepted methods of analysis differed internationally include immunoassay, mass spectrometry, and liquid chromatography (Table 2). Standardization of laboratory assays is an issue of controversy. All societies except the CMHF endorse the consensus statement from the CDC that states many factors can confound results (Old AUA: n/a, 2018 AUA: n/a, AACE: n/a, Endocrine Society: 1; +++)[10-15]. There is not yet a national standard for

collecting and analyzing samples in the United States, however, several groups supporting the consensus statement are working towards hard cut offs. The AUA, Endocrine Society and the CMHFsupport the CDC testosterone standardization program which certifies commercial testosterone assays[11, 12, 14, 15]. In contrast, Europe has a national external quality control program in place that serves to improve consistency of results across labs[32].

Adjunctive testing

TheCMHF, AACE, old AUA, and EAU guidelines, recommend free testosterone and sex hormone binding globulin (SHBG) analysis in those with equivocal total testosterone (CMHF: Strong recommendation; Moderate evidence,AACE: n/a, Old AUA: n/a, EAU: A; 1)[8, 9, 11, 33]. In men who have conditions that alter SHBG or those with initial total testosterone measurements near the lower limit of normal, the Endocrine Society and older AUA guidelines recommend testing ofalbumin (Endocrine Society: 1; +++, old AUA: n/a)[11, 15]. The 2018 AUA **does not recommend free** testosterone measurements as the primary diagnostic method for TD due it its high co-efficient of variation[2, 14, 34]. However, the Panel does recognize that free testosterone measurement may have utility in patients with total testosterone levels in the low/normal or equivocal range[14].

With regard to adjunctive testing after initial diagnosis the EAU and CMHFrecommends that hypogonadism be differentiated into primary and secondary types with the utilization of LH levels (EAU: B, 1b; CMHF: Strong recommendation; Moderate evidence)[8, 9]. The Endocrine Society and AACE

recommends FSH in addition to LH measurement to aid in differentiation between types of TD (Endocrine Society: 1; +++, AACE: n/a)[10]. The AACE further suggests that dynamic tests such as GnRH stimulation testor prolactin, be conducted and interpreted by an endocrinologist in patients with persistent borderline values of gonoadotropins. The older AUA guidelines do not mention differentiation, while the 2018 guidelines make extensive recommendations on adjunctive testing including LH, and prolactin. (Strong recommendation; Grade A)[14]. In men with total testosterone less than 150ng/dL with low or low normal LH they suggest ordering pituitary MRI *regardless* of prolactin levels in order to detect non-secreting adenomas[14]. Similarly, AACE and Endocrine Society recommend a prolactin level and pituitary imaging in men with acquired hypogonadotropic hypogonadism[10, 15]. The Endocrine Society also recommends karyotyping in men with primary hypogonadism with unknown origin and with a testicular volume < 6 ml to assess for Kallmann Syndrome(2; ++)[15].

CONCLUSION

TD affects multiple organ systems and can have a variety of health consequences. The causes of TD are wide-ranging, thus the diagnosis and management of TD has historically proven to be problematic for clinicians. The objective of this review was to shed light on the consensus statements between the American, Canadian, and European guidelines on how to identify and evaluate TD.

All organizations conclude that TD should be evaluated when the history and physical, along with any potential laboratory results, seem to indicate

possible TD. Specific populations who can be considered for diagnostic evaluation of TD differ between societies, with the EAU specifying the greatest number of special populations while the Endocrine Society and the AACE specify the least. There isa consensus that questionnaires are unreliable and should be avoided.

When labs are drawn for evaluation of TD a total and free testosterone is recommended by all societies, while free testosterone measurement is not recommended by 2018 AUA. Method of analysis for testosterone levels differed between societies, however immunoassay, mass spectrometry, and liquid chromatography were the most commonly recommended. None of the guidelines agreed on a low testosterone cutoff value. Recommendations for adjunctive testing varied across guidelines and should be aimed towards differentiating between primary and secondary causes of TD.The TD guidelines reflect differences in world health care systems and these differences highlight the need for further research to elucidate best evidence based practices in the evaluation and treatment of TD.

Table 1.WHEN TO EVAUALUATE: Populations recommended for diagnostic evaluation for possible testosterone deficiency by Canada, Europe,AUA, Endocrine Society, and the AACE

Populations for consideration	Canada	Europe	United States			
			Old AUA	2018 AUA	Endocrine Society	AACE
Historical findings	x	x	x	x	x	x
Clinical findings	x	x	x	x	x	x
Anemia	x			x		
Sarcopenia	x	x				
Refractory depression	x					
HIV	x	x		x		
Glucocorticoid use	x	x		x		
Opioid therapy/chronic opioid use	x	x		x		
ED with PDE-5 inhibitor failure	x					
Obesity		x				
Metabolic syndrome		x				
Diabetes type 2		x		x		
ESRD on dialysis		x				
Pituitary dysfunction				x		
Radiated pituitary mass		x				
Moderate-severe COPD		x				
Osteoporosis/Bone density loss		x		x		
Chemotherapy exposure				x		
Testicular radiation				x		
Infertility		x		x		

Key:
x- indicated in guidelines
HIV- human immunodeficiency virus ED- Erectile dysfunction
PDE-5- phosphodiesterase type 5 inhibitor
ESRD- End stage renal disease COPD- chronic obstructive pulmonary disease

Table 2.DIAGNOSIS OF TD:Analysis and comparison of lab testosterone cut off values, sample collection timing, delay of collection due to illness, preferred analysis methodology, and second line confirmatory testing that are currently recommendedin guidelines by Canada, Europe, and America.

		Canada	Europe	United States			
				Old AUA	2018 AUA	Endocrine Society	AACE
Diagnostic Recommendations	Low T cutoff (ng/dL)	N/A	Total T <348 Free T<18.8	No rigid cutoff; Total T <300 Free T <6.5	Total T <300 Free T <6.5	Total T<264	Total T <200
	Laboratory collection	Morning, within 3hrs awakening, fasting	Morning, fasting	Between 7-11am Night shift adjustments	Morning, fasting	Morning, fasting	Morning, fasting
	Delay collection if ill	n/a	n/a	x	x	x	n/a
	Preferred analysis methodology	IA MS	IA MS	Variable	IA MS LC	IA LC tandem	Equilibrium dialysis Free T Index
	Second line confirmatory testing	Free T or SHBG in patients with equivocal Total T	Free T or SHBG in patients with Total T 230-345ng/dL	Free T or SHBG in patients with equivocal Total T	No Free T	Free T in patients with equivocal Total T	Free T or SHBG in patients with equivocal Total T

Key:
n/a- not mentioned in recommendations x-indicated in guidelines
LC- liquid chromatography IA- immunoassay
MS- mass spectrometry T- testosterone
SHBG: sex hormone binding globulin

Appendix 1. Level of Evidence Definitions (adapted from AUA, CMHF, EAU, and AACE)

AUA	2018 AUA	CMHF	EAU	AACE	Endocrine Society
N/A	A: High certainty Benefit>Risk/burden or vice versa Net benefit or harm is substantial Applies to most patients andin most circumstances and future research is unlikely to change confidence	High Quality: Consistent evidence from RCTs or strong evidence from unbiased observational studies	1a: Evidence obtained from meta-analysis of randomized trials Ib: Evidence obtained from at least one randomised trial.	N/A	++++: High quality Consistent evidence from RCTs or strong evidence from unbiased observational studies
N/A	B: Moderate certainty Benefit>Risk/burden or vice versa Net benefit or harm is substantial Applies to most patients andin most circumstances but better evidence could change confidence	Moderate Quality: Evidence from RCTs with important limitations (inconsistent results, methodological flaws, indirect or imprecise evidence) or unusually strong evidence from unbiased observational studies	2a: Evidence obtained from one well-designed controlled study without randomization 2b: Evidence obtained from at least one other type of well-designed quasi-N/A experimental study	N/A	+++: Moderate quality of evidence Evidence from RCTs with important limitations (inconsistent results, methodological flaws, indirect or imprecise evidence) or unusually strong evidence from unbiased observational studies
N/A	C: Low certainty Benefit>Risk/burden or vice versa Net benefit or harm appears substantial Applies to most patients andin most circumstances but better evidence is likely to change confidence	Low Quality: Evidence for at least one critical outcome from observational studies, from RCTs with serious flaws, or indirect evidence	3: Evidence obtained from well-designed non-experimental studies, such as comparative studies, correlation studies and case reports 4. Evidence obtained from expert committee reports or opinions or clinical experience of respected authorities	N/A	++: Low quality of evidence Evidence for at least one critical outcome from observational studies, from RCTs with serious flaws, or indirect evidence
N/A	N/A	Very Low	N/A	N/A	+: Very low

		Quality: Evidence for at least one of the critical outcomes from unsystematic clinical observations or very indirect evidence			quality evidence Evidence for at least one of the critical outcomes from unsystematic clinical observations or very indirect evidence

Appendix 2. Grade Definitions (adapted from AUA, CMHF, EAU, and AACE) *note2018 AUA guidelines do not mention “grade” but do have nomenclature modifying statement type to evidence of strength which was adapted for this table

AUA	2018 AUA	CMHF	EAU	AACE	Endocrine Society
N/A	Strong recommendation: Net benefit or harm substantial	Strong recommendation: based on the quality of the supporting evidence, the level of uncertainty between desirable and undesirable clinical effects or diagnostic reliability, and therapeutic preferences.	A: Based on clinical studies of good quality and consistency addressing the specific recommendations and including at least one randomized trial.	N/A	1: Strong recommendation Used to modify LOE Benefit clearly greater than harms/burden or vice versa
N/A	Moderate Recommendation: Net benefit or harm moderate	N/A	B: Based on well-conducted clinical studies, but without randomized clinical trials	N/A	N/A
N/A	Conditional Recommendation: No apparent net benefit or harm	Weak Recommendation: based on the quality of the supporting evidence, the level of uncertainty between desirable and undesirable clinical effects or diagnostic reliability, and therapeutic preferences.	C: Made despite absence of directly applicable clinical studies of good quality	N/A	2: Conditional recommendation Used to modify LOE Benefit closely balanced with harms/burden Requires more carefulconsideration of the circumstances, values, and preferences to determine the best course of action
N/A	Clinical Principal: Component of clinical care that is widely agreed upon by clinicians for which there may or may not	N/A	N/A	N/A	N/A

	be evidence in the medical literature				
N/A	Expert Opinion: achieved by consensus of the Panel, that is based on members' clinical training, experience, knowledge, and judgment for which there is no evidence	N/A	N/A	N/A	Ungraded Good Practice Statement: Direct evidence for thesestatements was either unavailable or not systematically appraised. Intention is to draw attentionand remind providers of these principles

References

1. Bhasin, S., et al., *Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline.* J Clin Endocrinol Metab, 2010. **95**(6): p. 2536-59.
2. Araujo, A.B., et al., *Prevalence of symptomatic androgen deficiency in men.* J Clin Endocrinol Metab, 2007. **92**(11): p. 4241-7.
3. Wang, C., et al., *Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA and ASA recommendations.* Eur J Endocrinol, 2008. **159**(5): p. 507-14.
4. Nieschlag, E.B., HM.; Nieschlag, S., *Testosterone: action, deficiency, substitution.* Cambridge University Press, 2004.
5. Bassil, N., S. Alkaade, and J.E. Morley, *The benefits and risks of testosterone replacement therapy: a review.* Ther Clin Risk Manag, 2009. **5**(3): p. 427-48.
6. Lee, Y., *Androgen deficiency syndrome in older people.* J Am Assoc Nurse Pract, 2014. **26**(4): p. 179-86.
7. Kaufman, J.M. and A. Vermeulen, *The decline of androgen levels in elderly men and its clinical and therapeutic implications.* Endocr Rev, 2005. **26**(6): p. 833-76.
8. Morales, A., et al., *Diagnosis and management of testosterone deficiency syndrome in men: clinical practice guideline.* CMAJ : Canadian Medical Association Journal, 2015. **187**(18): p. 1369-1377.
9. G.R. Dohle (Chair), S.A., C. Bettocchi, and S.K. T.H. Jones, M. Punab, *Guidelines on Male Hypogonadism*, in *European Association of Urology*. 2015. p. 24.

10. Petak, S.M., et al., *American Association of Clinical Endocrinologists Medical Guidelines for clinical practice for the evaluation and treatment of hypogonadism in adult male patients--2002 update.* Endocrine Practice: Official Journal Of The American College Of Endocrinology And The American Association Of Clinical Endocrinologists, 2002. **8**(6): p. 440-456.
11. Paduch, D.A., et al., *The laboratory diagnosis of testosterone deficiency.* Urology, 2014. **83**(5): p. 980-8.
12. Rosner, W. and H. Vesper, *Toward excellence in testosterone testing: a consensus statement.* J Clin Endocrinol Metab, 2010. **95**(10): p. 4542-8.
13. Directors, A.U.A.B.o., *AUA Position Statement on Testosterone Therapy.* 2014.
14. Mulhall JP, T.L., Brannigan RE, Kurtz EG, Redmon JB., Chiles KA, Lightner DJ, Miner MM, Murad MH, Nelson CJ, Platz EA, Ramanathan LA, Lewis RW *Evaluation and Management of Testosterone Deficiency: AUA Guideline.* J. Urol, 2018.
15. Bhasin, S., et al., *Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline.* J Clin Endocrinol Metab, 2018.
16. Guyatt, G.H., et al., *Guideline panels should not GRADE good practice statements.* J Clin Epidemiol, 2015. **68**(5): p. 597-600.
17. Wu, F.C., et al., *Identification of late-onset hypogonadism in middle-aged and elderly men.* N Engl J Med, 2010. **363**(2): p. 123-35.
18. Mulligan, T., et al., *Prevalence of hypogonadism in males aged at least 45 years: the HIM study.* Int J Clin Pract, 2006. **60**(7): p. 762-9.
19. Millar, A.C., et al., *Predicting low testosterone in aging men: a systematic review.* Cmaj, 2016. **188**(13): p. E321-30.
20. Araujo, A.B., et al., *Prevalence and incidence of androgen deficiency in middle-aged and older men: estimates from the Massachusetts Male Aging Study.* J Clin Endocrinol Metab, 2004. **89**(12): p. 5920-6.
21. Harman, S.M., et al., *Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging.* J Clin Endocrinol Metab, 2001. **86**(2): p. 724-31.
22. Bhasin, S., et al., *Testosterone dose-response relationships in healthy young men.* Am J Physiol Endocrinol Metab, 2001. **281**(6): p. E1172-81.
23. Bhasin, S., et al., *Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham Heart Study and applied to three geographically distinct cohorts.* J Clin Endocrinol Metab, 2011. **96**(8): p. 2430-9.
24. Vesper, H.W., et al., *Interlaboratory comparison study of serum total testosterone [corrected] measurements performed by mass spectrometry methods.* Steroids, 2009. **74**(6): p. 498-503.
25. Brambilla, D.J., et al., *The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men.* J Clin Endocrinol Metab, 2009. **94**(3): p. 907-13.

26. Paduch, D.A., et al., *Testosterone Replacement in Androgen-Deficient Men With Ejaculatory Dysfunction: A Randomized Controlled Trial.* J Clin Endocrinol Metab, 2015. **100**(8): p. 2956-62.
27. Muehlenbein, M.P., et al., *Toward quantifying the usage costs of human immunity: Altered metabolic rates and hormone levels during acute immune activation in men.* Am J Hum Biol, 2010. **22**(4): p. 546-56.
28. Nankin, H.R. and J.H. Calkins, *Decreased bioavailable testosterone in aging normal and impotent men.* J Clin Endocrinol Metab, 1986. **63**(6): p. 1418-20.
29. Carani, C., et al., *Effects of androgen treatment in impotent men with normal and low levels of free testosterone.* Arch Sex Behav, 1990. **19**(3): p. 223-34.
30. Baskin, H.J., *Endocrinologic evaluation of impotence.* South Med J, 1989. **82**(4): p. 446-9.
31. Demisch, K. and T. Nickelsen, *Distribution of testosterone in plasma proteins during replacement therapy with testosterone enanthate in patients suffering from hypogonadism.* Andrologia, 1983. **15 Spec No**: p. 536-41.
32. Stavelin, A., et al., *An overview of the European Organization for External Quality Assurance Providers in Laboratory Medicine (EQALM).* Biochemia Medica, 2017. **27**(1): p. 30-36.
33. Hubert Vesper and J.C. Botelho, *CDC LABORATORY/MANUFACTURER HORMONE STANDARDIZATION (HOST) PROGRAM STANDARDIZATION OF SERUM TOTAL TESTOSTERONE MEASUREMENTS*, L.Q.A.a.S. Programs, Editor. 2013, Center for Disease Control and Prevention.
34. Brand, J.S., et al., *Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies.* Int J Epidemiol, 2011. **40**(1): p. 189-207.