LABORATORY DIAGNOSIS OF TESTOTERONE DEFICIENCY

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XVI th World Meeting of Sexual Medicine

Sao Paulo, 8-12 October 2014

Jacques Buvat: disclosure slide

- Advisor: Eli Lilly, Nextmed, Johnson et Johnson
- Speaker: Eli-Lilly, Janssen, Menarini, Nextmed,
- Investigator: Bayer, Boehringer-Ingelheim, Janssen, Lilly,

Definition of Testosterone Deficiency (TD)

Buvat et al 2013, ISSM recommendations

- Testosterone Deficiency is a clinical AND biochemical syndrome, frequently associated with age and co-morbidities, and characterized by a deficiency in testosterone and relevant symptoms.
- Low T may result from defects at various levels of the hypothalamus- pituitary- testes axis:
  - In the testes (primary TD)
  - At pituitary or hypothalamic level: secondary or tertiary TD
- TD may also result from an impairment of T action:
  - Decreased bioavailability of T, due to SHBG variations
  - Decreased sensitivity to T due to Andr. Receptor alterations

Laboratory Diagnosis is indicated in presence of symptoms, signs and conditions indicative of TD

Buvat et al 2013

Most specific signs and symptoms	Less specific signs and symptoms	Most specific Conditions
Reduced sexual desire and activity	Decreased energy, motivation, initiative	Type 2 diabetes mellitus
Decreased spontaneous erections	Retarded ejaculation	Metabolic syndrome
Erectile Dysfunction	Reduced muscle bulk and strength	Chronic obstructive lung disease, Sleep Apnea Syndrome
Hot flushes, sweats	Diminished physical or work performance	End-stage renal disease, hemodialysis
Waist circumference > 102 cm Increased BMI	Mild anemia (normocytic, normochromic)	Osteoporosis
Decreased testes size Varicocele	Depressed mood, irritability	HIV- associated weight loss
Loss of pubic hair, reduced requirement for shaving	Poor concentration and memory	History of infertility, cryptorchidism, pituitary disease, delayed puberty
Height loss, low trauma fractures, reduced BMD	Sleep disturbances, sleepiness	Treatment with opioids or glucocorticoids

But no indication for routine measurement of T

Methods to measure testosterone

LABORATORY TEST	ADVANTAGES	DRAWBACKS
Total testosterone (TT): Liquid chromatography/ mass spectrometry (lcms)	Reference method Most accurate, especially for low values	More expensive Available in few laboratories (ref labs)
Immunoassays:RIA Chemiluminescence	Less expensive More widely available	Less accurate, especially when TT in the low range
Free testosterone (FT): Equilibrium dialysis (ED)	Gold standard Most accurate	Expensive, labor inten- sive, limited availability.
Analogue immunoassay:	Less expensive Easily accessible	Not correlated with ED NOT RECOMMENDED
cFT: calculated with T and SHBG	Good correlation to equilibrium dialysis. Less expensive	Need accurate TT (lcms) and SHBG. Right formula debated*.
Bioavailable T (BT): Ammonium-sulf. precipit.	Measures 1-2% FT + 30-35% albumin-bound	Expensive, labor inten- sive, prone to errors.
cBT: calculated with T and SHBG	Less expensive	Overrates the BT level NOT RECOMMENDED

*** Ly et al 2010, Mazer 2010**

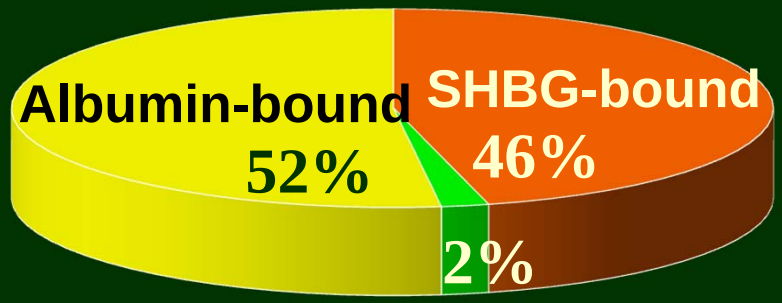
FREE TESTOSTERONE HYPOTHESIS

Free Testosterone:
= non protein-bound T
= sole bioactive part ??

Bioavailable Testosterone

= free + alb.-bound T

=non SHBG-bound T



The free hormone hypothesis may be a gross over simplification whose validity has not been established beyond a reasonable doubt (*R Ekins, I990, J Faix, 2013*).

Today generally accepted, though no evidence of a better association of TD symptoms with FT compared with TT.

Clinical conditions and hormones variations that affect the SHBG circulating level, and hence the circulating fractions of T in men

SHBG increase Free and bioavailable T decrease	SHBG decrease Free and bioavailable T increase
Aging	Obesity
Hepatic diseases Cirrhosis	Insulin resistance Hyperinsulinism
Hyperthyroidism	Hypothyroidism
Estrogens	Androgens
Tamoxifen	Hyperprolactinemia
Anti-epileptic drugs	GH, Acromegaly
	Corticosteroids, Hypercorticism

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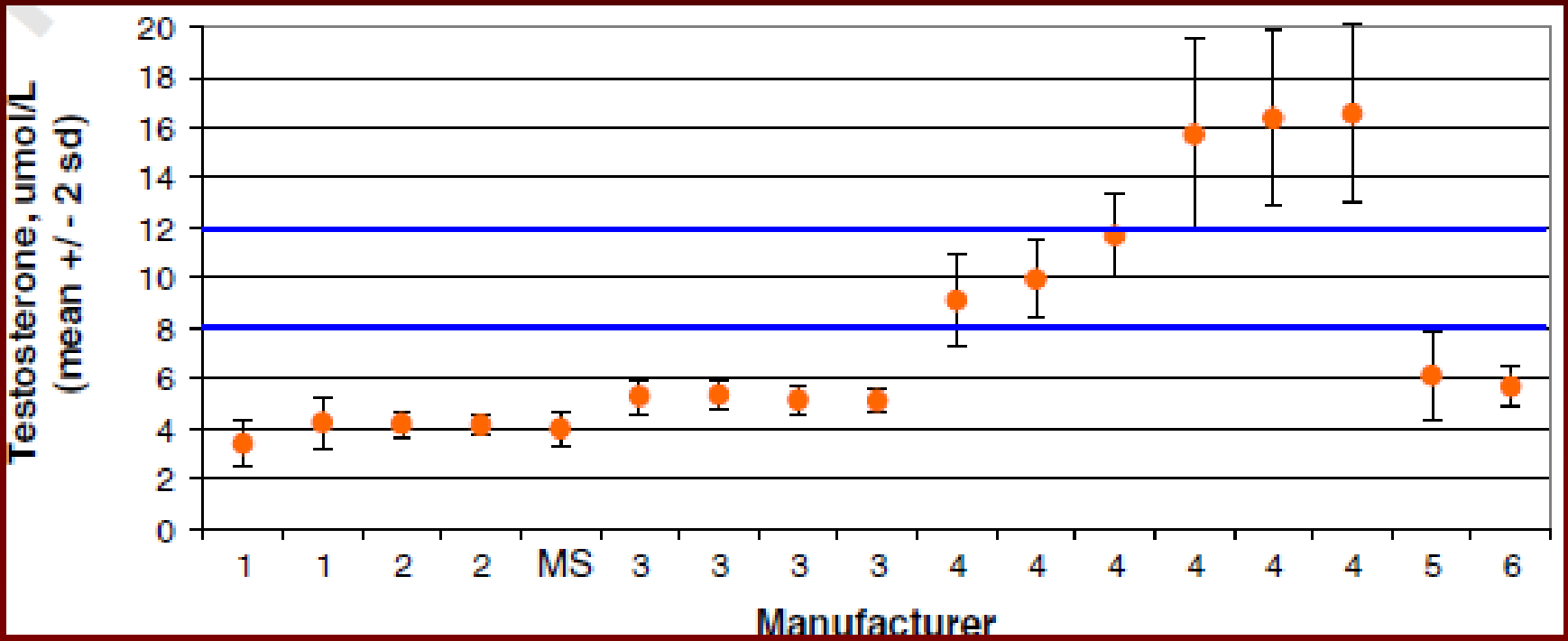
Should LCMS replace immunoassays in routine practice? *(Fanelli et al 2013)*

- Since the 90's, major concerns about accuracy of IAs used in most routine labs, especially for low T concentrations

Variability of the results of TT measurements according to the immunometric platform *(Morales 2012)*

Results of TT measurement in the same serum sample provided to 1500 labs according to the 17 platforms supplied by 6 manufacturers that these labs were using (College of American Pathologists' Ligand Special External Proficiency Testing Program)



T concentrations are generally consistent in the same manufacturer, but not across manufacturers, though population references ranges were similar

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Should LCMS replace immunoassays in routine practice? *(Fanelli et al 2013)*

- LC has improved the practicability of MS, the Gold Standard technology, with respect to Gas Chromat.
- LCMS demonstrated its analytical superiority over immunometric platforms in the reference labs
- Reduces but does not eliminate interlab variations *(Vesper et al 2009, Owen et al 2012, 2013, Rhea et al 2013)*, and requirement for control groups in each lab
- Requires also validation and more standardization
- Specific drawbacks: high initiation and maintenance costs, requirement of highly experimented staff

Comparison of Mass Spectrometry and Immunoassays in reference laboratories

- A validated IA platform is sufficient to differentiate hypo versus eugonadal males *(Wang et al 2004, Huhtaniemi et al 2012),* while not reliable in women and children

- With respect to MS, T measured with IA provides clinically relevant info as concerns
  - sexual desire, body composition, grip strength *(Araujo et al 2013)*
  - cardiometabolic risk factors *(Haring et al 2013)*

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Liquid Chromatography Tandem Mass Spectrometry vs Immunoassay: conclusions

- LCMS is now the reference method which may allow a definitive standardization of T measurements and the generation of widely accepted reference intervals, hence a consensus on utility of biochemical testing
- But presently not justified to substitute it to IAs for the diagnosis of hypogonadism in routine practice
- Today the real issue is to improve the reliability of IAs:
  - by using LCMS to standardize the variety of IAs commercially available to routine clinical labs
  - by obtaining from clinical labs implementation of published guidelines as concerns traceability and participation in external quality assessment programs

Some reference values established with with LCMS

Author Journal	Reference population	Mean total T ± SD (ng/ml)	2.5th percentile	Mean FT ± SD (pg/ml)	2.5th percentile
Bhasin JCEM 2011	456 healthy men <40 y	7.23 ± 2.2	3.48 ng/ml 12 nmol/l	141.8 ± 45	70 pg/ml 242 pmol/l
Yeap JCEM2012	394 healthy older men 76.1±5.2 y	4.06 ± 1.38	1.84 ng/ml 6.4 nmol/l	56.8 ± 29.9	14.7 pg/ml 103 pmol/l

Should we measure TT or FT?

- No evidence supports an advantage to measure FT rather than TT. Based on theoretical grounds
- Very few doctors have access to equilibrium dialysis
- Analogue FT assay not recommended
- Calculated FT or BT may seem a convenient issue. In fact it is a complicated way for non endocrinologists
  - Requires going to internet to find a calculator
  - Issue of the units (nmol or ng, /ml, dl or l)
  - No reference range
- Scientific validity of cFT and cBT debated

Controversies around using cFT or cBT

- **Criticisms against Vermeulen's formula for cFT**
  - **Albumin variations disregarded: but little impact** ***(Guay et al 2013)***
  - **Erroneous constants used in the equation?**
    - **4 other equations proposed, resulting in slight changes in the FT level** ***(Ho et all 2006, Ly et al 2010, Guay et al 2013)***
  - **SHBG polymorphisms may affect binding affinity** ***(Ohlsson et al 2011)***
- **Prevalent opinion: FT is a supplementary method. Not to be used alone**

Comparison of methods for assessing T concentration *(Araujo et al, ENDO 2013)*

Comparison of 7 methods for assessing T

- CI Chemiluminescence IA
- LCMS Liquid chromatography tandem mass spectrometry
- Bioavailable T by ammonium sulfate precipitation (BAT)
- Calculated BT with CI
- Calculated FT with CI & LCMS
- Bioactive assay

For their ability to predict 4 androgen-sensit. endpoints

- Isometric grip strength
- Lean mass & fat mass
- Hematocrit
- Sexual Desire Inventory

- In 795 randomly selected men 35-80 y

Comparison of methods for assessing T: CONCLUSIONS *(Araujo et al, ENDO 2013)*

- **Correlations of T with androgen sensitive end-points vary by age and by obesity**
- **In their reference center IA and LCMS performed similarly**
- **Consideration of SHBG aids interpretation, particularly in older or obese men**
- **But calculation of FT confers no additional useful information:**
  - **cFT had the lowest correlation with androgen-sensitive EPs**
  - **Was no more informative in older or obese patients**

At what time of the day should we measure T?

- **Early morning preferable in young men due to T nycthemeral variation. Later gives falsely low results**
- **After 40 attenuation diurnal variation *(Luboshitzky et al 2003, Brambilla et al 2009)*, hence less risk of false diagnosis**
  - **Several retrospective studies with clear limitations give inconsistent results:**
    - ✓ **no influence of draw-time on mean TT values till 02pm *(Crawford et al 2007, Guay et al 2008, Welliver et al 2014),* then decrease by about 13% *(Crawford et al 2007).***
    - ✓ **decrease of mean FT and BT end morning compared with early morning *(Guay et al 2008)***
  - **1 prospective study: At any age 50% of men with a low TT in the afternoon have normal values in the morning *(Brambilla et al 2009, small series)***
- **At any age, morning is preferable, especially for FT/BT**

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Should we measure Dihydrotestosterone or Dehydroepiandrosterone (DHEA)?

- No
- Serum DHT levels do not reflect the DHT production within the target tissues, which is the most important
- No benefit of supplementation with DHEA in controlled studies

Does measuring serum LH add to the diagnosis of TD?

- **Yes in men with a low or borderline TT at a first measurement: high or high normal LH:**
  - **Definitely confirms hypogonadism, and its primary (testicular) origin, thus to be treated with testosterone itself**

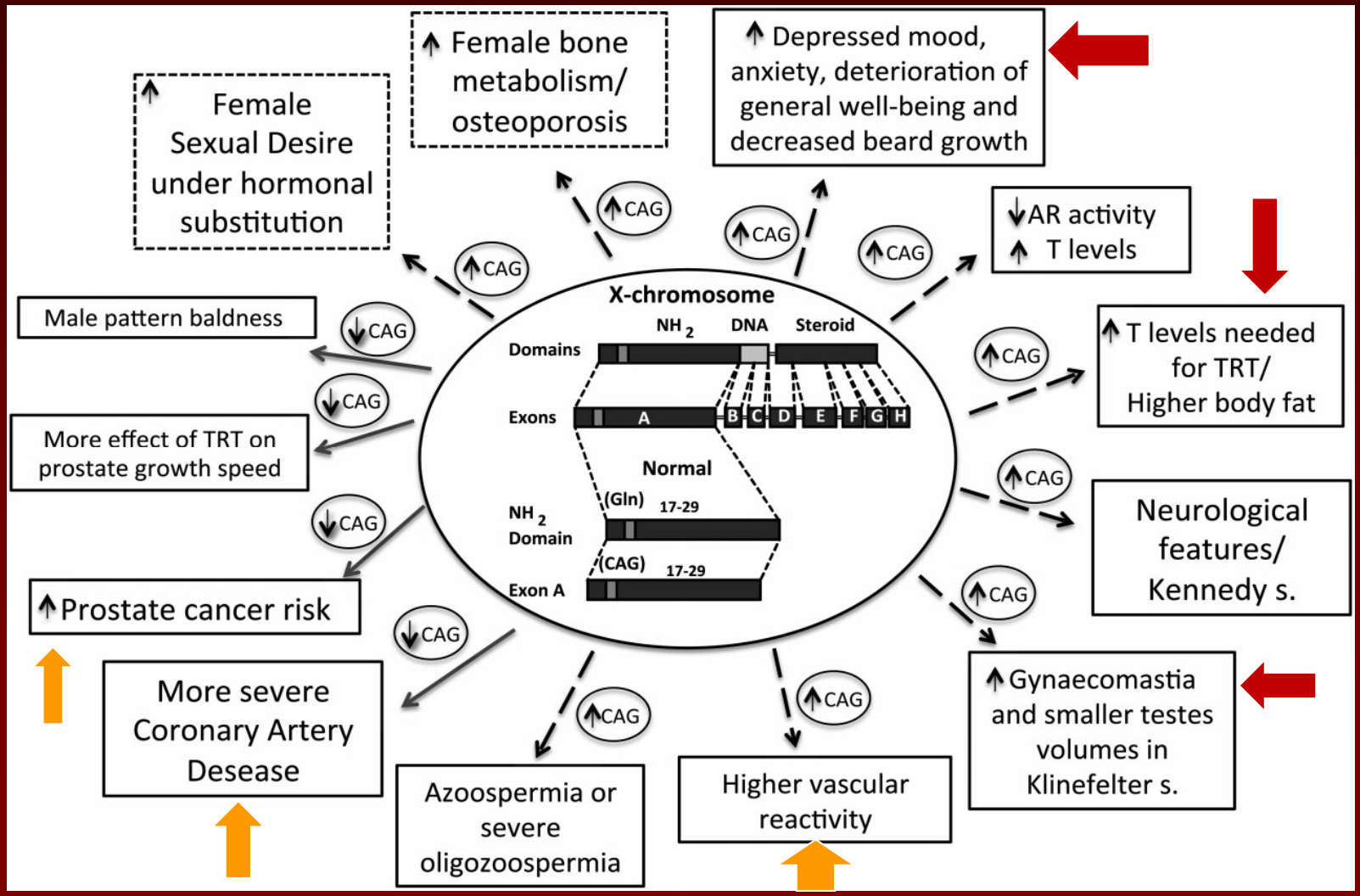
- **Probably no in association to the 1st T measurement:**
  - **« subclinical », or « compensated hypogonadism » (high LH + normal T)**
    - **is prevalent in older men (9.5% after 40, 21% in 70-79 years old, *Tajar et al 2011, EMAS*)**
    - **Is associated with ⇧ likelihood of physical but not sexual symptoms *(Tajar et al 2011)***
    - **May be a forerunner of overt primary hypogonadism**
    - **But no available evidence data on efficacy and safety of T therapy *(Tajar et al 2011, Gianetti et al 2013)***

Is CAG repeat testing necessary for the best management of TD?

- Serum T level only partly reflects the androgenic status: polymorphisms of the AR gene, especially the number of CAG repeats in exon 1 modulate its transcriptional activity *(Zitzmann 2009)*
  - This may be determined by PCR then automatic DNA sequencing
  - The longer the CAG repeats' length, the lower the AR efficiency, causing significant decreases of androgenicity in eugonadal men and of efficacy of T therapy in certain studies
  - This may at least partly account for the interindividual variability of the sensitivity to T

Associations of CAG repeat polymorphism with clinical features linked to TD

(Francomano et al, 2013)



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Is CAG repeat testing necessary for the best management of TD? *(Francomano et al 2013)*

- In some men TD symptoms might result from ⇧ length of CAG repeats, despite only borderline T levels
- ⇧CAG repeatslength predicts incident low T *(Haring 2011)*
- But investigationnal studies' results are inconsistent
  No definite TD symptoms in all men with longer repeats
  (Schneider 2010,2011,2013, Jozkow 2010, Nenonen 2010, Andersen 2011, Ferlin 2011,Stanworth 2011, Sankar 2012, Folland 2012,Tirabassi 2013,14
- May be due in part to a compensatory ⇧ in T (and E2)
  (Lapauw 2008, Huthaniemi 2009, Travison 2010)
- Knowledge still too preliminary and inconsistent to support a diagnostic interest at this stage. May become important in a near future. A role in future management of T therapy is also probable

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Strategy for the laboratory diagnosis of Testosterone Deficiency (Grade C)

- In patients suspected of TD, these investigations are recommended:
  - Morning serum sample for Total Testosterone (TT) determination.
  - In case of a low level it is recommended:
    - to repeat the TT determination (LOE3)
    - together with serum LH, and prolactin measurements (LOE1)
    - and in case of only moderately low or borderline TT, SHBG if alterations of its circulating level are suspected (especially in obese or older men) (LOE3).

Results' interpretation

- **Repeat T measurement: if first T result moderately low, repeat testosterone is normal in 1/3 cases *(Buvat et al 2013)***
- **Serum LH:**
  - **Elevated: primary/testicular TD**
  - **Elevated + normal or borderline T: subclinical TD, may be symptomatic *(Ucak et al 2013)***
  - **Low or normal: secondary TD. Possility of modifiable causes [hyperprolactinemia (HPRL) or hemochromatosis]**
  - **Very low LH and T: think to pituitary tumor, even if normal PRL**
- **Serum prolactin (PRL)**
  - **HPRL classical cause of TD. LSD & ED may be revealing sympt**
  - **In men, pituitary tumor in 50% of non iatrogenic severeHPRLs**
  - **MRI if PRL ≥ 35 ng/ml**
- **T: what is a low testosterone level ??**

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What is a low testosterone level? Using one or several threshold levels?

- Endocrine Society: threshold # 3 ng/ml, 11.4 nmol/l
- Does not take into account the variability of the sensitivity to circulating T:
  - Interindividual variability *(Kelleher et al 2004)*
  - Variability according to the symptom: different T thresholds for different tissues and symptoms *(Gray et al 2005, Zitzmann et al 2006, Wu et al 2010)*
  - Variability according to age (tends to ⇘) *(Gray et al 2005)*
  - Interlaboratory variability of the results *(Sikaris et al 2005, Morales 2012)*
  - Variability of sensitivity of the AR *(Zitzmann et al 2008)*
- Difficult to propose only one cut-off value which could not integrate all these individual variations

Threshold levels for the laboratory diagnosis of TD (Grade B)

- There are no generally accepted lower limits of normal TT. There is, however, general agreement that:
  - TT > 12 nmol/l (3.5 ng/ml) does not usually require substitution (LOE1)
  - Based on the data of young hypogonadal men, men with TT < 8 nmol/l (2.3 ng/ml) usually benefit from T treatment (LOE1)
  - Between these levels
    - measuring SHBG levels may be helpful. Referral to an endocrinologist may be of help in case of substantially abnormal level
    - A trial of testosterone therapy may be envisaged for 6-12 months in those patients who are symptomatic, while alternative causes of the symptoms have been excluded (LOE2) Beyond that time, T therapy would be continued only in case of substantial benefit (LOE2)

We have now evidence of beneficial effects of T therapy in men with TT between 8 and 12 nmol/l

Study Journal	Formulation Duration	Mean T * nmol/l	Significantly improved parameter, p
Allan, 2008, IJIR**	Patches 12 months	13.6/13.5	IIEF sexual desire score, +1.18/pl group, p=0.04
Srinivar-Shankar 2010, JCEM	Gel 6 months	10.9/11	Isometric knee extension torque, p=0.02
Giltay, 2010, JSM	Nebido 30 weeks	8 ± 4	IIEF 5: +3.1/pl group, p<0.001
Hackett, 2013, JSM	Nebido 30 weeks	9.2/8.9	IIEF EFD score: +2.82, p<0.001
Hackett, 2014, IJCP, mild group	Nebido 30 weeks	8-12	Waist circumf.: -3.5 cm, HADS, both <0.001, HbA1c :-0.24 p=0.03, CT: -0.23, p=0.02

* Mean TT at baseline, active/placebo groups, ** high drop-out rate

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The trial of T therapy: a simple clinical diagnostic test in symptomatic patients

- In men with TT between 8 and 12 nmol/l (2.3-3.5 ng/ml, 230-350 ng/dl)
- Exclude contraindications
- Objective assessment of signs and symptoms (weight, WC, metabolic tests, AMS, IIEF)
- After initiation of T therapy control that the targetted T level has been achieved
- Follow-up every 3 or 6 months with the same tests
- After 6 to 12 months, T therapy would be continued only in case of substantial benefit

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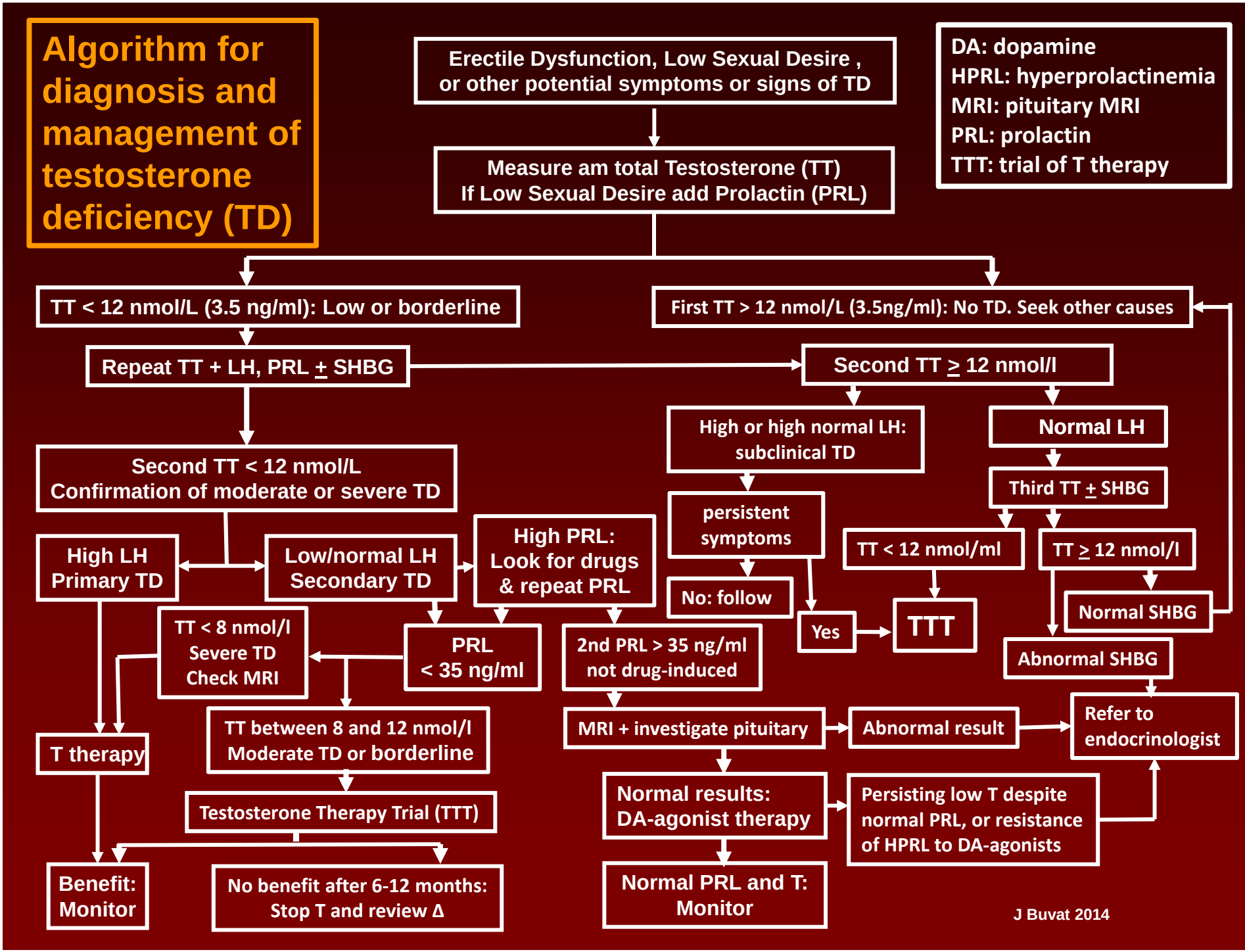
Algorithm for diagnosis and management of testosterone deficiency (TD)
Erectile Dysfunction, Low Sexual Desire , or other potential symptoms or signs of TD
Measure am total Testosterone (TT)
If Low Sexual Desire add Prolactin (PRL)
DA: dopamine
HPRL: hyperprolactinemia
MRI: pituitary MRI
PRL: prolactin
TTT: trial of T therapy
TT < 12 nmol/L (3.5 ng/ml): Low or borderline
First TT > 12 nmol/L (3.5ng/ml): No TD. Seek other causes
Repeat TT + LH, PRL ± SHBG
Second TT ≥ 12 nmol/l
Second TT < 12 nmol/L
Confirmation of moderate or severe TD
High or high normal LH: subclinical TD
Normal LH
Third TT ± SHBG
persistent symptoms
TT < 12 nmol/ml
TT ≥ 12 nmol/l
High LH
Primary TD
Low/normal LH
Secondary TD
High PRL:
Look for drugs
& repeat PRL
No: follow
Yes
TTT
Normal SHBG
TT < 8 nmol/l
Severe TD
Check MRI
PRL
< 35 ng/ml
2nd PRL > 35 ng/ml
not drug-induced
Abnormal SHBG
T therapy
TT between 8 and 12 nmol/l
Moderate TD or borderline
MRI + investigate pituitary
Abnormal result
Refer to endocrinologist
Testosterone Therapy Trial (TTT)
Normal results:
DA-agonist therapy
Persisting low T despite normal PRL, or resistance of HPRL to DA-agonists
Benefit:
Monitor
No benefit after 6-12 months:
Stop T and review Δ
Normal PRL and T:
Monitor
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