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Testosterone deficiency syndrome: Treatment and cancer risk☆

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ABSTRACT

Testosterone deficiency syndrome (TDS) can be linked to premature mortality and to a number of co-morbidities (such as sexual disorders, diabetes, metabolic syndrome, . . .). Testosterone deficiency occurs mainly in ageing men, at a time when prostate disease (benign or malign) start to emerge. New testosterone preparations via different route of administration appeared during the last decade allowing optimized treatment to these patients. One potential complication of this treatment is the increased risk of prostate and breast cancer. Consequently, the guidelines from the agencies and the institutions, the recommendations of the scientific expert committees and the attitude of the clinicians to who, when and how to treat hypogonadal patients, is very conservative, not to say, highly restrictive. To date, as documented in many reviews on the subject, nothing has been found to support the evidence that restoring testosterone levels within normal range increases the incidence of prostate cancer. In our experience, during a long-term clinical study including 200 hypogonadal patients receiving a patch of testosterone, 50 patients ended 5 years of treatment and no prostate cancer have been reported. In fact, the incidence of prostate cancer in primary or secondary testosterone treated hypogonadal men is lower than the incidence observed in the untreated eugonadal population. However, even if the number of patients treated in well-conducted clinical trials for whom cancer of the prostate has been reported is insignificant (a very few), the observed population is still too small to raise definite conclusions. Low testosterone levels have been reported in patients undergoing radical prostatectomy and the outcomes are of worse diagnostic in this population; at a later stage, testosterone deficiency can be induced by anti hormonal manipulation of patient with a prostate cancer, leading to the symptoms of hypogonadism. The question is to know whether it is justified, in case of profound symptoms, to supplement those patients with testosterone. Some attempts have been made and the results are encouraging: so it is time to re-examine our position and to question about the definite recommendation that patients with prostate cancer should never receive testosterone supplementation therapy; this is already the situation when intermittent androgen blockade is initiated if the biological response is satisfactory. Furthermore, it has been advocated that, under a rigorous surveillance, patients cured of prostate cancer can be treated with testosterone supplementation with beneficial results.

1. Introduction

For more than 50 years, treatment of male primary (testicular failure or resulting from testicular trauma) or secondary (pituitary or hypothalamic failure) hypogonadism is common and well established. Even if the decline of testosterone with age is very slow, a significant number of men over 50 years have a biological testosterone below the normal range. This could be due to aging, obesity, metabolic syndrome, severe systemic illness such as AIDS, cirrhosis or medication interfering with the biosynthesis of testosterone such as antiandrogens, corticoids, LHRH analogues. The treatment of that testosterone deficiency is not really accepted. Hypogonadism affects an estimated 2–4 million men in the United States, of which only 5% receive treatment [1].

The reluctance to treat patients is essentially based upon a risk assessment. Should we treated symptomatic patients when we do not know to what extent testosterone decline is associated with clinically important sequelae when it is believed by the majority of urologists that testosterone treatment increases prostate cancer risk. It is difficult to argue that testosterone supplementation is a safe treatment when no long-term study in a large population, followed for many years, do not simply exists. The possibility of increasing prostate cancer incidence in an aging population without proven benefits has become a major issue. In 2002, the Institute of Medicine (IOM) has nominated a committee on assessing the need for clinical trials of testosterone replacement therapy [2].

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However, during the last decade, following the introduction of new delivery testosterone formulations (patch, gel, long acting injection), clinical investigators have found that the benefits overcome the risks. There is ample reason and evidence to treat men with testosterone who have signs and/or symptoms of hypogonadism [3]. New questionnaires have been developed to facilitate the detection and the characterization of symptoms caused by testosterone deficiency [4–6]. Guidelines and statements have been issued by various associations and societies (Endocrine Society [7], American Society of Andrology, International Society of Andrology, International Society for the Study of Aging Male, European Association of Urology, European Academy of Andrology) and updated. The more recent revised recommendations, written by expert representatives of the various institutions, are published simultaneously in the *Journal of Endocrinology*, *European Urology*, *International Journal of Andrology*, *International Journal of Impotence Research*, *Journal of Andrology*, and *The Aging Male* [8].

It is a consensus that "testosterone therapy for symptomatic men with androgen deficiency, who have low testosterone levels induce and maintain secondary characteristics and improve their sexual function, sense of well-being, muscle mass and strength, and bone mineral density". If the patient is hypogonadal and symptomatic, testosterone treatment will be useful; however, there is no reason to give testosterone to asymptomatic and eugonadal patients; this should be considered as steroid abuse and is a regulating bodies issue.

The Canadian Society for the study of the aging male created an International Consultation Group [9] and then send a letter to Health Canada in which they stated that "it would be discriminatory to deny to symptomatic hypogonadal men the right to government (or private) insurance benefits for testosterone treatment if, in the judgment of the treating doctor, such therapy was deemed to be appropriate based on generally accepted standards of practice. Men requiring, or at least candidates for testosterone therapy, should be treated no differently from men or women who receive support for treatment of any kind upon reasonable medical assessment. The Group agreed that long term double blinded placebo-controlled studies in large numbers of men were needed to lay to rest concerns about efficacy of testosterone treatment, but that short-term studies, the long history of testosterone use, and clinical experience when considered together provide more than adequate evidence to treat carefully selected hypogonadal men using the currently available international guidelines and recommendations as a basis for the management of men who are potential candidates for such treatment".

They stated that "at present time, there is no conclusive evidence that testosterone therapy increases the risk of prostate cancer or Benign Prostatic Hyperplasia (BPH). There is also no evidence that testosterone treatment will convert sub-clinical prostate cancer to clinically detectable prostate cancer. However, there is unequivocal evidence that testosterone can stimulate growth and aggravate symptoms in men with locally advanced and metastatic prostate cancer. Prior to therapy with testosterone, a man's risk of prostate cancer must be assessed using, as a minimum, digital rectal examination and determination of serum prostate-specific antigen (PSA). Pre-treatment prostate ultra-sound examinations or biopsies are not recommended as routine requirements. After initiation of testosterone treatment, patients should be monitored for prostate disease at 3–6 months, 12 months, and at least annually thereafter". Although there is no causal relationship between testosterone and prostate cancer, it is advisable for medical–legal reasons that, before initiating testosterone therapy, an informed consent, in which the benefits and risks are carefully explained, should obtained from the patient [10].

2. Epidemiological evidence of a correlation between testosterone and prostate cancer?

During the last decade, numerous epidemiological studies and several reviews have tried to delineate the relationship of serum sex hormone concentrations and prostate cancer.

In 1996, Gann et al. [11] observed in The Physician's Health study, in which 22,071 physicians have identified 520 cases (222 over 10 years follow-up), a significant increase of risk of prostate cancer with higher testosterone levels (highest quartile vs. lowest testosterone OR = 2.36) after simultaneous adjustment for sex hormone binding globulin (SHBG) and estradiol.

In 1997, Vatten et al. [12] reported in the Linkage of Norwegian National Cancer Registry (approximately 28,000 men with blood samples; 59 incident prostate cancer cases and 180 controls identified between 1973 and 1994), no difference in testosterone levels between groups, no increased risk of cancer with increased quartile of testosterone and no trend of risk with increasing testosterone levels.

In 1999, Eaton et al. [13] concluded from a meta-analysis that there was some overall evidence for a positive association of an end-product of dihydrotestosterone (DH)T metabolism, androstanediol-glucuronide (Adiol-G), with prostate cancer risk suggesting that increased rate of intra-prostatic conversion of testosterone into DHT, using Adiol as an alternative marker of prostatic DHT, might indeed enhance prostate cancer development. Kaaks et al. [14] pointed out that, in several of the cohort studies, prostate cancer risk was associated with levels of serum DHT, although not significantly; circulating DHT level is not a reliable indicator of DHT formation because circulating DHT is also largely formed in the skin. Heikkila et al. [15] found in a longitudinal study (166 cases of prostate cancer, 300 controls; maximum 24 years follow-up), a mild increase (RR = 1.27 (0.67–2.37)) for highest/lowest testosterone comparison.

In 2000, Shaneyfeld et al. [16] performed a meta-analysis of all previously published studies on hormonal predictors of risk for prostate cancer. They found that no steroid hormone seems to be significantly associated with the development of prostate cancer. But, when restricted to studies that performed mutual adjustment for testosterone, DHT, estradiol, age, and body mass index (BMI), men whose testosterone is in the highest quartile, are 2.34 [1.30–4.20] times more likely to develop prostate cancer. Neither DHT nor estradiol seems to be associated with the development of prostate cancer. Hoffman et al. [17] retrospectively reviewed the clinical record of 117 consecutive patients with prostate cancer, in one center. Fifty-seven underwent radical prostatectomy. Patients with low free testosterone have more extensive disease and all men with a biopsy Gleason score > 7 had low serum free testosterone suggesting that low free testosterone may be a marker for more aggressive disease.

In 2001, Mohr et al. [18] published the results of a well-documented prospective study from the Massachusetts Male Aging Study (MMAS). In a sample of 1576 men, 70 were diagnosed with prostate cancer during the 8 years by follow-up period. They were 5 years older but had no differences in BMI, alcohol use, and smoking. No significant differences between cases and non-cases were found for any hormones in the unadjusted analyses and the age-adjusted logistic revealed that none of the hormones were associated with prostate risk at the 0.01 level with the exception of Adiol-G. These observations seem to indicate that high conversion of testosterone to DHT as observed following oral administration or injection of testosterone esters could increase the risk of prostate cancer. Hsing [19] reviewed 12 modest-sized prospective studies where the association between circulating levels of androgens with prostate cancer risk has been inconclusive.

In 2004, Stattin et al. [20] reported a large prospective study of more than 200,000 men with data coming from the Nordic Biological Specimen Biobank Working Group (Norway, Finland and Sweden). The authors found mild, albeit significant, decreases in risk for increasing levels of testosterone down to an OR for top vs. bottom quintile of 0.80 [0.59–1.06] (p trend 0.05) and a corresponding OR for SHBG of 0.76. There is no difference in the cohorts in the three countries. Investigation of the risk associated with extreme levels of testosterone (2-fold difference) on prostate risk remained unchanged. The authors concluded that in a search for hormonal changes and genetic polymorphisms associated with prostate cancer risk, changes affecting metabolism downstream of testosterone are the ones most likely to yield positive findings. It is worth to note that, in this largest longitudinal study published so far, an increased risk of cancer of the prostate was observed with low testosterone levels. This is in accordance with Hoffman et al. [17].

In 2005, Isom-Batz et al. [21] reviewed retrospectively 3890 patients with clinically localized prostate cancer: out of them, 326 testosterone levels were measured. Testosterone was a predictor of pathological stage: as testosterone decreased, the likelihood of organ confined disease decreased.

In 2006, the results of The Melbourne Collaborative Cohort Study of 17,049 men ages between 27 and 75 have been published by Severi et al. [22]. At diagnosis, the mean age of the patients was 67 years, 430 had non-aggressive and 88 aggressive cancer. There was little evidence that levels of androgens influenced overall risk of prostate cancer: the linear trends in the Hazard Ratio values (HR represent the relative difference in risk associated with a doubling of the concentration) for testosterone, DHEAS, androstenedione and free testosterone differed significantly between aggressive and non aggressive cancer. The risk of aggressive prostate cancer significantly decreases with increasing levels of testosterone, DHEAS and androstenedione (p trends between 0.005 and 0.03). These findings do not support the long prevailing "androgen hypothesis" that high levels of circulating androgens increase prostate cancer risk. Instead, high levels within the reference range of androgens, estrogens and adrenal androgens decrease aggressive cancer prostate risk.

Rhoden and Morgentaler [1] concluded that the clinical implications of this type of data analysis was uncertain; Shaneyfeld et al. [16] stated that, because serum sex steroids are interrelated, adjustment for mutual confounding is of critical importance; Imamoto et al. [23] pointed out the limitations of these studies including retrospective designs and diurnal variation in androgens which were not account for in these studies. Most investigators assessed steroid hormone concentrations at one time point in midlife, although the etiologically relevant time period is unknown.

The difficulties in demonstrating positive associations between serum androgen levels and prostate cancer in epidemiological settings can partly be explained by several methodological limitations, including limited statistical power in most studies, the relatively small number of incident cases in follow-up studies (>150 cases), the relative small differences (10–15%) in mean serum levels of hormones between cases and controls, and the somewhat large laboratory variations in intra- and inter-assays of serum hormones. The variation of methods between laboratory with different sensitivity, specificity and storage conditions is a main concern before drawing definite conclusion from epidemiological studies. Appropriate hormone assays should be used in accordance with the Endocrine Society position statement [24]: it has not been always the case, thus all meta-analysis performed so far in this domain have many biases and limitations.

The best functional testosterone seems to be the bio-available testosterone (free + albumin bound) [25]. Bio-available testosterone can be estimated from total testosterone and SHBG using a simplified formula derived from the law of mass action [26]. Vermeulen et al. [27] have developed a calculator which is proposed on the web site of ISSAM. Calculated free testosterone or bio-available testosterone has become one of the biochemical parameters to characterize hypogonadism and is included in the recommendations particularly in the obese men [8].

We have conducted, during the last years, clinical studies with a transdermal testosterone delivery system [28]. It is well known that free testosterone cannot be measured either by an analog displacement [29,30] or by equilibrium dialysis (too labor-intensive for a great number of blood samples). We have chosen to measure bioavailable testosterone following ammonium sulfate precipitation, by the same technologist in the same laboratory, and to validate the assay. By comparing the measured values with the calculated values, we found a 2-fold higher calculated value and we questioned about the general use of the calculator for taking a clinical decision based upon not appropriately defined reference range concentrations [31].

We then analyzed in depth this discrepancy and found that the association constant for SHBG and albumin were inappropriate and that the serum concentration of androstenediol, which decreases markedly with age, has to be taken into account. The association constant has been adapted to age and the most appropriate adjusted affinities for SHBG were $K_s = 1.9 \times 10^9$ L/mol instead of 1.0×10^9 L/mol and for albumin was $K_a = 2.45 \times 10^4$ L/mol instead of 3.6×10^4 L/mol [32]. In these conditions, calculated bio-available testosterone values were superimposed to assayed values. The calculation is not straightforward and is highly sensitive to variations in the affinities constant which have to be calibrated in each laboratory with assayed bio-available testosterone.

In our opinion, the use of a simplified formula is inappropriate and assays of testosterone before and after precipitation by ammonium sulfate in the same laboratory should allow a better evaluation and comparison of the results coming from different studies.

In this context, we cannot give full credit to the conclusions in the report of Parsons et al. [33] who found in the Baltimore Longitudinal Study of Aging, that higher levels of calculated serum free and free testosterone index are associated with an increased risk of prostate. In this study, including 794 and 2958 samples of community-dwelling men over a period of 40 years, there was no significant age-adjusted association of serum total testosterone, DHEAS or SHBG with prostate cancer and tumor grade. Unfortunately, DHT and AdiolG are not given.

From these epidemiological prospective studies and some others non cited here, and the partial unsuccessful attempts to consolidate the results of some of them by meta-analysis, the Endogenous Hormones and Prostate Cancer Collaborative Group has been created with the aim to examine in the existing worldwide epidemiologic data, the association between sex hormones and the risk of prostate cancer [34]. Eighteen prospective studies (representing more than 95% of the worldwide published data) including 3886 men with incident prostate cancer and 6438 control subjects were pooled. Relative risks of prostate cancer by fifths of serum hormone concentration were estimated. The main finding of this study was that pre-diagnostic serum concentrations of testosterone, free testosterone, DHT, Adiol-G, DHEA-S, androstenedione, estradiol, or free estradiol were not associated with the risk of subsequent prostate cancer. If the concentration of serum androstanediol glucuronide is a valid marker of intraprostatic activity, such activity is not related to the risk of prostate cancer. A modest inverse association between serum SHBG concentration and prostate cancer risk has been noted. The authors recognize that this study has several limitations (methodology, surveillance of the patients, a single hormone assay) which are largely described but these limitations seem not to affect the conclusions to a significant extend. However, they weaken the conclusions by a process similar to a regression to the mean. The null finding on total testosterone derived from

the analysis was predictable because, in the majority of the studies pooled, there was no clear or no association at all between hormone concentrations and prostate cancer risk.

In line with the comments of Hsing et al. [35], we can conclude that the null finding on total serum testosterone from the pooled analysis underscores the importance of a better understanding of the relationship between tissue and serum levels of androgens to clarify the role of androgens in prostate cancer. Possibly, prostate cancer risk is related more on intra-prostatic concentration of DHT and depends more upon variations in intra-prostatic DHT formation than on fluctuations in circulating total testosterone.

By searching on the Medline database indexed on testosterone, Calof et al. [36] extracted 19 studies and showed that testosterone replacement in older men was significantly associated with higher risk of detection of prostate events than placebo-treated men. However, this analysis assumed that each prostate event occurred in a separate individual (same person might have more than one prostate event), overestimating total prostate event rates. A further analysis of prostate events reveals significant bias that could contribute to the increase of events in testosterone-treated men. Prostate biopsies in testosterone trials are often triggered by an increase of PSA levels, thus testosterone-treated men are more likely to undergo biopsy. As a significant proportion of older men have sub-clinical prostate cancer, a greater number of prostate biopsy would likely led to the detection of greater number of sub-clinical prostate cancer.

On the basis of this meta-analysis, Calof et al. have determined sample size estimates for detecting increase in prostate cancer rates in testosterone-treated men compared with placebo-treated men. This analysis indicates that 85,862 participants would be needed in each group to detect an increase of 20% in prostate cancer rates in testosterone-treated men compared with placebo-treated men. Forty thousand men would be needed to detect a 30% increase, and 8591 men would be needed in each group to detect a 70% increase, if treatment duration were 1 year. These sample size estimates could be affected significantly by treatment duration. As studies of this magnitude would require substantial resources, the IOM Expert Panel's recommendation to focus initially on efficacy trials seems prudent and realistic.

We urgently need a well-conducted international trial with an accepted protocol designed by a scientific board of experts recruited in basic science and clinical research, associated to methodologists and statisticians and, accepted by the Institutions. Until results could be available, we have to inform and educate clinicians with the last advances in this field, in order to consolidate their own judgment. It has been a mistake to leave this global approach to pharmaceutical companies when we see the consequence on patients, clinicians and authorities of the Women Health Initiative [37] and of the Prostate Cancer Prevention Trial [38].

3. Androgens and prostate

Androgens are necessary for the development and normal function of the human prostate. Consequently, a causal relationship between androgenic hormones and human prostatic carcinogenesis is plausible because prostate carcinoma develops from an androgen-dependent epithelium and is usually androgen-sensitive at early stage of the disease. The transcription of genes involved in growth and survival of the prostate cells depends primarily on the androgen receptor, which is activated by DHT. Androgenic action in the prostate is function of androgen receptor concentration and its co-regulators, and tissue concentration of DHT. DHT is formed within the prostate from testosterone and from androstenedione via 5α-androstanedione and inactivated through 3α and 3β-androstanediol metabolites which are glucuro-conjugated and irreversibly eliminated.

Androgens could induce prostate neoplastic growth through various pathways: circulating androgens, androgen-metabolizing enzyme (reductase, aromatase, hydroxy-steroid-dehydrogenase). Changes in the signaling of the androgen receptor pathway can occur form alterations in concentration of the androgen within the cancer cell, transformation of receptor gene or mRNA (shorter CAG repeat lengths confer greater activity of the androgen receptor and have been associated with an increased risk and earlier onset of cancer [39]) alterations in the receptor function, modifications in the co-regulator and co-repressor molecules, or androgen-independent activation of the receptor itself [40,41].

Androgen deprivation strategies lead to depletion of testosterone, inactivation of the androgen receptor, or both, and finally, senescence of many cancer cells. The production of PSA is androgen-regulated and undergoes a sharp decline following surgical or medical castration. PSA is specifically useful in assessing the response to local therapies and the most common form of treatment, androgen deprivation therapy [42]. Furthermore, PSA gene expression may be independent of androgens and promoted by vitamin D, IL-6, growth factors and protein kinases [43].

In the absence of androgens, the tumors evolve into castrate-resistant phenotypes led by changes in the androgen receptor. Mutations, nucleotide polymorphisms of CAG regions on exon1 of the androgen receptor gene, co-regulation of gene amplification, and cross-talk with peptide growth factors (TGFβ, EGF, IGF1, ...) that stimulate the transcription of androgen-responsive genes in a ligand androgen independent manner have been described [43].

Manipulation of the androgen milieu has to be conducted with precaution, keeping in mind that our knowledge is too limited to decide definitively of the standard treatment for androgen deficiency and for prevention of prostate tumors. In the Prostate Cancer Prevention Trial [38], the reduction by 25% of the risk of prostate cancer by finasteride supports the critical role of DHT in the prostate but the concomitant increase of high-grade prostate cancer is a strong incentive for a conservative approach. Results from this prospective study suggest that pharmacologically lowering DHT formation might favour the development of aggressive prostate cancer. Nishiyama et al. [44] put into evidence in 47 patients, the association between the DHT level in the prostate and prostate cancer aggressiveness. DHT has been measured in prostatic tissue and in serum. DHT in prostatic tissue in patients with Gleason scores 7–10 was significantly lower than in those with Gleason score 6 or less ($p = 0.025$) This result has many implications, one of them is to give an explanation to the proliferation of aggressive prostate cancer in a low DHT environment, as it is the case with finasteride and potentially dutasteride [45] medication.

4. Prostate cancer aggressiveness and low testosterone levels

Pre-treatment testosterone in patients with prostate disease has been intensively investigated during the last decade. Studies have consistently demonstrated that low testosterone implied more aggressive disease, worse prognostic and worse treatment response. Schatzl et al. [46] have investigated whether low serum testosterone levels in men with newly diagnosed prostate cancer have an association to the endocrine status, PSA levels, Gleason score, and receptor expression. In 156 newly diagnosed cancer patients, 52 had an androgen deficiency (serum testosterone level < 3 ng/mL) with significant lower estradiol, LH and FSH (secondary hypogonadism) but a significant ($p = 0.0001$) higher Gleason score (7.4 vs. 6.2). The mean testosterone levels decreased from 4.1 (1.7) ng/mL in patients with Gleason score < 5 to 2.8 (2.7) ng/ml with Gleason score > 8. In agreement with Hoffman et al. [17] and Isom-Batz et al. [21], Massengill et al. [47] found on a larger cohort of 879 patients treated with radical prostatectomy that patients

with low serum testosterone had more positive surgical margins. Teloken et al. [48] have evaluated, in a transversal study, the association between serum total testosterone levels and found that pre-operative low serum testosterone (<2.7 ng/mL) can predict a significant increase in positive margins in patients with localized prostate cancer who underwent radical prostatectomy.

Garcia et al. [49] published a cross-sectional study including 31 prostate cancer patients and 25 gender-matched controls of similar age. They found no significant difference in testosterone levels between the 2 groups: 11.8 ± 0.62 nmol/L, in the cancer group and 13.41 ± 0.94 nmol/L, in the control group. However, SHBG levels were significantly higher in the cancer group (175 ± 18 and 101 ± 8 nmol/L; $p = 0.001$) and albumin levels were lower 3.38 ± 3.01 and 3.9 ± 0.26 g/dL; $p < 0.001$). Calculated free testosterone levels were decreased in the cancer group 0.08 ± 0.01 nmol/L vs. 0.14 ± 0.01 nmol/L in the control group as well as calculated bio-available testosterone 1.44 ± 0.15 nmol/L vs. 3.01 ± 0.24 nmol/L; $p < 0.001$. Serum LH was elevated in cancer patients, suggesting a primary testicular dysfunction. BMI was not correlated to SHBG in the control group and was inversely correlated with SHBG in the cancer group.

Mearini et al. [50] reported an open clinical study including 128 patients who referred to the Department of Urology of the University of Perugia because of the onset of lower urinary tract symptoms or accidental finding of high PSA levels (63 patients had BPH and 65 prostate cancer). The mean serum testosterone concentrations were significantly higher in patients with BPH than in those with prostate cancer (4.7 ng/mL vs. 3.3 ng/mL, $p < 0.005$). Moreover, 37% of the patients with prostate cancer had testosterone levels below normal (<2.5 ng/mL) and 26% reached the castration threshold (<0.5 ng/mL). Only 9% of the patients with BPH had testosterone levels below normal. Testosterone clearly emerged as a marker of prognosis. In patients who underwent radical prostatectomy, the pre-operative testosterone levels correlated with tumor stage, as determined on the basis of surgical samples, suggesting the lower testosterone concentration, the higher the probability of finding advanced-stage disease. Indeed, at the testosterone cutoff value <2.5 ng/mL, 75% of the patients had locally advanced or metastatic prostate cancer. Consequently, the baseline testosterone concentrations appear to be worth to be included among other, well-accepted and widely used parameters for prognosis. A testosterone value <2.5 ng/mL increases predictive accuracy of the tests and assumes an independent predictive value. Multivariate analysis in patients with prostate cancer showed that the lower the testosterone concentration, the more advanced the disease is.

Imamoto et al. [23] reported that low pre-treatment serum testosterone levels had a significant predictive value for higher stage prostate cancer in 82 patients with clinically localized prostate cancer. Lackner et al. [51] have found that in 126 prostate cancer patients, men with high-grade cancer (Gleason 7–10) had lower levels of serum testosterone than did those with low-grade cancer (3.49 ng/mL vs. 4.09 ng/mL; $p = 0.056$). Hypogonadal men had a greater Gleason score than eugonadal men.

Raynaud and Botto [52] presented at the 2008 ESSM/ISSM meeting, the results of a study aimed to characterize the relationship between the aggressiveness of the tumor assessed by the Gleason score and the pre-operative testosterone serum concentration. One hundred and thirteen consecutive prostate cancer patients, who underwent radical prostatectomy in the Department of Urology of the Hospital Foch (France) during the year 2007, had a pre-operative testosterone determination. Blood samples were assayed for total testosterone and bio-available testosterone by a validated RIA method. Gleason score was determined in prostate biopsies and radical retropubic prostatectomy (RRP) specimens by the same uro-pathologist. Patients were stratified in 2 groups; patients with a Gleason score of 6 (3 + 3) or 7 (3 + 4) and patients with a Gleason score of 7 (4 + 3) or 8 (4 + 4). Mean total testosterone levels were 5.12 ng/mL ($N = 82$) and 3.62 ng/mL ($N = 31$), in groups 1 and 2, respectively ($p = 0.004$). For bio-available testosterone, mean bio-available testosterone level was 1.12 ng/mL ($N = 82$) and mean bio-available testosterone level was 0.8 ng/mL ($N = 31$), in groups 1 and 2, respectively ($p = 0.043$).

Thus, low testosterone could cause prostate cell deregulation and predisposes the prostate to aggressive neoplastic changes. There is some evidence that obese men have decreased risk for low-grade cancer and increased risk for high-grade disease [53]. In this population testosterone serum level is generally low.

Daniell [54] analyzed the survival after orchiectomy for 78 men with prostate cancer in relation with testicular atrophy. He found highly undifferentiated tumors (Gleason scores 8–10) more often present in the 37 men with testicular atrophy at the time of orchiectomy compared with the 41 men without testicular atrophy (30% vs. 10%; $p < 0.03$) and concluded that these results supports a worse prognosis for men with atrophy at the time of orchiectomy. Multiple studies documenting a similarity worse prognosis for hypogonadal men receiving chemical androgen ablation suggest that hypogonadism also may anticipate a poor prognosis in men receiving other forms of therapy for their prostate cancer.

The measurement of testosterone level before any treatment of cancer of the prostate should be added to PSA determination for the prognosis and during the follow-up of the treatment.

5. Testosterone replacement therapy: biological effects

The use of testosterone preparation, mainly the parenteral form, to treat primary or secondary hypogonadal men is well established for many decades and is still the only recognized reference for the Drug Agencies. The advent of new delivery forms, taking advantage of the transdermal route to avoid liver first pass-effect and to allow a better safety, has opened new avenues to treat testosterone deficient patients whatever the cause. Coming first, is the population of ageing hypogonadal male denominated "late-onset of hypogonadism". Because, this population is at risk of prostate cancer, the International scientific community recommend to precisely delineate the symptomatic patient with a hormonal deficiency based upon a testosterone serum level determination. Testosterone is recognized to be the most important parameter for evaluation of effective substitution therapy of male hypogonadism [55]. However, this approach which has been extended to epidemiological studies has been very disappointing as presented by the pooled analysis discussed there above.

Behre et al. [56] conducted a three arm (hypogonadal, testosterone-treated hypogonadal and age-matched normal men) cross-sectional study to assess the effect of 6-month testosterone treatment (testosterone enanthate injections or testosterone scrotal patch, or testosterone undecanoaate orally) on prostate volume, PSA and hormone levels. They found that, in contrast to normal men in whom prostate volume increases significantly with age, the prostate of the untreated hypogonadal patients was significantly smaller and no significant correlation with age could be detected. When hypogonadal patients are treated with testosterone preparations, the prostate volume increased to the level that can be expected in normal men of comparable age. Testosterone serum levels were those of normal men but serum DHT levels were higher, particularly in the patients treated with the scrotal patch. However, the higher DHT levels were not associated with a greater increase of prostate volume. The peripheral serum DHT levels do not reflect the intra-prostatic tissue concentrations, as intra-prostatic DHT tissue concentration, is converted mainly locally to different metabolic products [57]. The consequence of Behre's study is that testosterone induced increase in prostate volume should not preclude hypogonadal men from necessary testosterone therapy.

Following this report, some attempts have been made to shed a light on the biological hormonal effect on the prostate tissue in men. A major advance was brought by Marks et al. [58] because of a significant improvement for androgen assays in prostate tissue (quick-frozen core biopsies [59]) for the analysis of prostate tissue specimen). They performed a randomized, double blind, placebo-controlled trial of 44 hypogonadal men (testosterone < 300 ng/dL and related symptoms), receiving either 150 mg testosterone enanthate or matching placebo intramuscularly every 2 weeks for 6 months. Screening testosterone levels were somewhat lower than baseline levels because the baseline levels were uniformly obtained between 8 and 12 a.m., while the screening levels were obtained at random times throughout the day. They have shown that exogenous testosterone, when administered for 6 months to men with symptomatic hypogonadism, in dosages sufficient to increase serum testosterone level to the mid-normal range (from a mean of 282–640 ng/dL), LH secretion was totally abolished. However, there was very little effect on the prostate gland. In particular, prostatic androgen levels were increased only slowly by testosterone replacement therapy. Additionally, prostate tissue composition and biomarkers of cell proliferation and angiogenesis were not altered, gene expression was not changed, and the occurrence of occult cancer was not increased.

The androgen-regulated biological functions in the prostate appear to be buffered against wide fluctuations in circulating androgens. The present study does not explain how this buffering mechanism works but explains that a saturation level exists for prostate tissue with regard to testosterone; when testosterone levels are higher, additional growth does not occur. Testosterone replacement therapy with a low dose in hypogonadal men caused prostate volume to increase to the size of age-matched but no more, even by increasing the dose.

Consequently, assay of testosterone and bio-available testosterone in serum and, testosterone and DHT in prostatic tissue or even other testosterone metabolites are necessary to further investigate associations between hormone levels and the prognosis for prostate cancer. A better understanding of the hormonal milieu within the prostate and the relationship with circulating hormones is the key issue to interpret results from serum-based studies and to expand our knowledge of the role of androgens in prostate cancer.

Concerning PSA levels, in the Mark's study [58] the PSA increase after 6 months as in Behre's study [56]. In the untreated hypogonadal group, PSA was significantly smaller than in the treated hypogonadal group, PSA levels were similar in both treated and normal men. This small increase during the first months of treatment has been observed in all the trials conducted so far with different exogenous testosterone treatment [60–66].

When testosterone is administered to young volunteers (mean 26 years) with increasing (100, 200, 500 mg/week) intramuscular testosterone, for 15 weeks, testosterone levels increased up to 20 ng/mL with no change in total and free PSA levels [67]. However, in this young population, PSA was low and not clinically representative of PSA levels in a clinically relevant group of men.

Gerstenbluth et al. [68] reported the results of a retrospective study of 54 hypogonadal patients (mean age 60.4 years; testosterone < 3.0 ng/mL) receiving testosterone intramuscularly (mean follow-up 30 months). Mean testosterone pre-treatment (1.89 ng/mL) went up during treatment to 9.74 ng/ml, in the upper physiological range. Mean pre-treatment PSA was 1.86 ng/mL (median 1.01) and increased to 2.82 ng/mL (median 1.5). One patient was diagnosed with prostate cancer. The authors concluded that testosterone therapy in men with erectile dysfunction and hypogonadism is associated with a minor PSA elevation. However, there does not appear to be a short-term increase for the development of prostate cancer.

Gould and Kirby [69] have made an excellent review on testosterone replacement therapy. Over a period of 15 years, 2200 men with symptoms of androgen deficiency were screened for prostate cancer and investigated for hypogonadism. Twelve had a prostate cancer, and 1500 were hypogonadal and treated. In this limited population, overall prostate cancer was 0.48% per year [64]. In Finland, 80,000 men were screened for prostate cancer and the overall prostate rate detection was 0.55% [70].

In their single centre clinical setting, McLaren et al. [71] performed a retrospective review of 85 patients with symptoms of testosterone deficiency. They observed that there was little change in the PSA values in patients continuing on testosterone replacement therapy over 2 years, despite increases in mean total testosterone or bio-available testosterone levels. No patients withdrew from testosterone replacement therapy because of exacerbation of any prostate related symptoms. During the present study, seven patients underwent eight prostate biopsies. Three patients were diagnosed with prostate cancer, one with high-risk localized disease (Gleason score 8), although this patient had been on testosterone therapy <1 year and had discontinued his therapy for >4 years, before his biopsy. All these observations are less than one would expect from the population under study.

Raynaud et al. [28], in the efficacy and safety study of a new testosterone-in-adhesive matrix patch applied every 2 days to hypogonadal men, have found no prostate cancer during the 5 years follow-up [72]. In this randomized, open label, multicenter European 5-year study, 224 hypogonadal patients were included (mean age 41.8 (12.4) years). One hundred and eighty eight patients received 2 patches of 60 cm^2 delivering 5 mg of testosterone daily, every 48 h and 36 patients had intramuscular testosterone enanthate injection every 3 weeks. After 1 year, all patients received patch only for four consecutive 1-year study extensions. Application of two transdermal patches of 60 cm^2 and their maintenance for 48 h leads to testosterone serum levels above normal range (3 ng/mL) in 85% of patients. Testosterone levels remained stable over time 5.8, 5.5, 5.3, 4.3, 4.4, 4.5, 4.9, 4.8, 4.5, 5.3 ng/mL. Serum PSA values showed a mean (SD) increase from baseline of 0.15 (0.42), 0.25 (0.92), 0.55 (0.88), 0.35 (1.66), 0.20 (0.53) and 0.15 (0.36) ng/mL at 3, 6, 9, 12, 18 and 24 months, respectively. The mean (SD) PSA velocity seemed to decrease over time (without considering three cases of prostatitis) with values of 0.043 (0.119) ng/mL/month at 6 months, 0.027 (0.138) ng/mL/month at 12 months, 0.011 (0.035) ng/mL/month at 18 months, and 0.006 (0.014) ng/mL/month at 24 months, and stabilized thereafter.

Several anecdotal reports have described development of prostate cancer after initiation of testosterone replacement therapy.

Curran and Bihrle [73] reported in an 85-year-old hypogonadal man with hyperlipidemia, coronary artery and peripheral vascular diseases, a 20-fold increase in PSA and a palpable prostate nodule, 6 months after the initiation of testosterone therapy with intramuscular testosterone. After cessation of testosterone, PSA felt down and then, the patient received LHRH analogue treatment.

Very few cases (less than 5) of prostate cancer in Klinefelter syndrome during hormonal replacement therapy have been reported. For instance, a patient with Klinefelter syndrome, who had undergone long-term (35 years) testosterone replacement therapy since childhood, had a localized prostate cancer (Gleason 6). He recovered after radical prostatectomy and was put again, 9 months later, under testosterone replacement therapy [74]. Another was a 55-year-old man who developed prostate cancer after 7 years of treatment [75]. Rhoden et al. [76] proposed to use the testosterone to PSA ratio as an independent and significant predictor of prostate cancer in hypogonadal men with a PSA < 4.0 ng/mL because, in this population, they found that the risk of prostate cancer was increased more than 3-fold when the testosterone-to-PSA ratio was <1.8.

Gaylis et al. [77] reported the medical records of 6 urology practices that identify 20 patients in whom prostate cancer developed while on testosterone therapy. Unfortunately, they cannot give the total number of men in order to know how common the risk of prostate cancer is in this setting.

We share the same opinion with Morgentaler [78] considering that these reports and their cautions regarding testosterone replacement therapy are examples of confirmation bias in which an observation seems to confirm a previously held belief without being subject to standard scientific rigor. It is true that testosterone is important for prostate cancer growth and that suppression of testicular androgen secretion by castration (surgical or medical) causes prostate cancer regression but it has never been either observed or demonstrated that raising testosterone in hypogonadal non castrated men leads to enhanced prostate cancer growth. What we have learn from prospective longitudinal studies is that men who develop prostate cancer do not have higher testosterone levels and when they have, the risk for developing prostate cancer is not greater than men with low testosterone concentration. Most important, physicians should be freed of antiquated and unscientific restrictions that inhibit optimal treatment of their patients.

The risk of exacerbation of occult cancer is always a key issue allowing to exclude from the treatment patient if any suspicion of prostate cancer, whatever the reason, is present. Even if it has been very elegantly shown that, after 1 year of testosterone treatment, men with prostatic intraepithelial neoplasia (PIN) do not have a greater increase in PSA or a significantly increased risk of prostate cancer than men without PIN, it is not generally accepted that testosterone is not contraindicated in men with a history of PIN [79].

6. Testosterone therapy and cancer of the prostate risk

Numerous studies have clearly demonstrated that testosterone has positive effects in attenuating the symptoms appearing during the aging process, particularly sexual and cognitive dysfunction, physical and behavioral capacity, metabolic syndrome [80]. Elucidation of the association between testosterone replacement therapy and prostate cancer is an important issue because of the large number of symptomatic hypogonadal ageing men who might potentially benefit from treatment.

It is taught, and permanently cited as an argument for androgen deprivation, that prostate cancer is not observed in eunuchs and that total androgen suppression by castration (surgical or chemical) is a first line treatment for advanced prostate cancer, when the tumor is still androgen-dependent. Indirect supports for the hypothesis that high levels of circulating androgens is a risk factor for prostate cancer have included the dramatic regression of tumor symptoms in a majority of men with advanced prostate cancer after castration. This is not relevant to the effect of variations within a physiologic range on early tumor events that takes place decades earlier. Indeed, there is also strong evidence that androgens could inhibit cancer cell growth by differentiation of the prostate epithelium.

Because the diagnostic of prostate cancer and testosterone replacement are common occurrences in urology practices, it is expected that some men receiving testosterone therapy are eventually likely to be diagnosed with prostate cancer. In fact, it has been reported a 29% incidence of occult prostate cancer in men older than 60 years with low serum total or free testosterone levels [81].

Clinicians and regulatory Agencies are concerned by the fact that testosterone therapy could cause or promote prostate cancer and are very reluctant to prescribe or to approve testosterone replacement treatment for the aging male. The IOM Committee on Assessing the Need for Clinical Trials of Testosterone Replacement Therapy in the US, recently concluded that insufficient evidence exists to justify embarking on a long-term study to determine the risks associated with testosterone replacement therapy [1]. Instead the committee recommended first performing short-term controlled studies of the effect of testosterone on several outcomes in elderly men whose testosterone levels were below 300 ng/mL. It is therefore, very doubtful that a definite answer will be forthcoming within the next 10–20 years, if ever.

In the meantime, hypogonadal symptomatic men should be considered for testosterone replacement therapy in line with published guidelines. It is important to exclude prostate cancer before initiation of treatment and to follow-up the patient with regular prostate monitoring. Patients should be informed that, to date, definitive placebo-controlled data relating testosterone treatment and prostate safety do not exist. A testosterone preparation that achieves physiological plasma levels without supra-physiological escape is preferred [8].

7. Testosterone therapy in prostate cancer patients

Fowler and Whitmore [82] have shown that previously untreated men with prostate cancer failed to demonstrate worrisome early progression with testosterone administration, for periods of up to several months. Some studies have recently appeared showing no adverse effects in hypogonadal men previously treated for a localized prostate cancer. Kaufman and Graydon [83], in a retrospective series of 7 hypogonadal men found that receiving androgen supplementation has been beneficial and safe. They concluded that hypogonadal patients with T1 or T2 disease, Gleason score < 8, pre-treatment PSA < 10 ng/mL and undetectable PSA after surgery are potential candidates for testosterone supplementation. These patients should be carefully counseled about the potential risk of testosterone treatment even if the prostate cancer is apparently cured, followed regularly for PSA and testosterone levels and if hypogonadal signs have improved, remain on testosterone replacement at the lowest dose necessary to be in the low-mid range of the physiological range. In case of a confirmed increase of PSA, the patient should discontinue immediately the testosterone supplementation.

Agarwal and Oefelein [84] have published the results of 10 patients who underwent radical retropubic prostatectomy for prostate cancer. Post-surgery mean PSA was <0.10 ng/mL and mean testosterone was 197 ng/mL. Patients received testosterone treatment for a median duration of 19 months. Testosterone increased significantly to 591 ng/mL. During the course of therapy no patient had PSA recurrence. The authors draw the same conclusions as Kaufman did. Furthermore, they suggested an interval between surgery and testosterone therapy >1 year.

To assess the risk of biochemical failure or prostate cancer recurrence, Sarosdy [85] has reviewed prospectively 31 patients who received testosterone supplementation after prostate brachytherapy with or without beam radiation therapy. The median serum PSA level was 5.3 ng/mL. Testosterone therapy was initiated from 0.50 to 4.25 years after brachytherapy and the median duration on treatment was 4.5 years. The decline of PSA after brachytherapy is slow and could be a problem to monitor these patients. However, the authors reported that no patients stopped testosterone treatment because of cancer recurrence or documented cancer progression.

After treatment for localized prostate cancer with external beam radiotherapy, five men with significant signs of testosterone deficiency were treated with testosterone (mean duration 14.5 months) once PSA level has reached the nadir; all patients reported an improvement in symptoms. The authors [86] concluded that there is a need for more information about the safety and efficacy of testosterone therapy in men successfully treated for localized

prostate cancer, because there is evidence indicating hypogonadism in these patients compromising their quality of life and longevity, independent of the cancer.

Despite the fact that these 4 studies are exploratory, performed in a small number of patients, non-randomized, without a placebo arm, they open a new field of clinical investigation to evaluate the feasibility of testosterone replacement therapy in patients with symptomatic hypogonadism after radical prostatectomy.

Because of the increase in life expectancy, an expanding population of patients successfully treated for prostate cancer will strongly desire receiving testosterone to prevent the devastating effects of hypogonadism [87]. They will be encouraged by a recent finding showing that testosterone insufficiency in older men is associated with increased risk of death over the following 20 years independent of multiple risk factors and severe preexisting conditions [88] and by the results of the European Prospective Investigation into Cancer in Norfolk [89] showing that in men, endogenous testosterone concentrations are inversely related to mortality due to cardiovascular disease and all causes. The analysis was insufficiently powered to examine the relationships with prostate or other specific cancer. However, an inverse relationship of endogenous testosterone concentration with cancer mortality was observed.

In a study aimed to survey the variations between different regions of the world in diagnosing and treating testosterone deficiency, physicians were interviewed [90]. The outcomes were that physicians require more education on diagnosing testosterone deficiency, on the role of testosterone in erectile dysfunction and the relative safety of testosterone treatment. We fully agree with the authors on the issue of "the fear that testosterone treatment of elderly men is associated with an increased risk of prostate cancer. This sentiment appeared stronger in Europe than elsewhere. In line with this, the readiness to prescribe testosterone more often, if it could be proven that this was safe, was greater in Europe than elsewhere. With the latest insight into the relationship between testosterone and prostate cancer, or prostate disease in general and the guidelines now in existence for prescribing testosterone to elderly men, these trepidations are no longer appropriate."

8. Conclusions

The traditional view that higher testosterone is a risk factor for prostate cancer is obsolete because of weak science-based evidences.

A better understanding of the hormonal milieu within the prostate and the relationship with circulating hormones is the key issue to interpret results from serum-based studies and to expand our knowledge of the role of androgens in prostate cancer.

Testosterone replacement therapy with a low dose in hypogonadal men caused prostate volume to increase to the size of age-matched but no more, even by increasing the dose. Thus, testosterone induced increase in prostate volume should not preclude hypogonadal men from necessary testosterone therapy.

The measurement of testosterone level before any treatment of prostate cancer should be added to PSA determination for the prognosis and during the follow-up of the treatment.

Men successfully treated for prostate cancer and suffering from confirmed symptomatic hypogonadism are potential candidates for testosterone substitution after a prudent interval if there is no clinical or laboratory evidence of residual cancer.

In any case, due to all these uncertainties, controversies and lack of expertise or knowledge of many physicians and investigators, men receiving testosterone therapy should be regularly monitored for prostate cancer.

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