REVIEW

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Variations of serum testosterone levels in prostate cancer patients under LH-releasing hormone therapy: an open question

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Abstract

The hypothesis 'the lower the better when achieving castration levels of testosterone' is based on the data from second-line hormonal manipulation and its molecular basis, and on better oncological results reported for lower castration levels in prostate cancer (PCa) patients, including those achieved with maximal androgen blockade. In this regard, the equivalence of surgical and different pharmacological castrations has been controversial. The modified amino acid structure that makes LH-releasing hormone (LHRH) analogs more potent than LHRH, and the method of delivering the analogs impacts on bioavailibility and potentially causes differences in androgen levels and in its final oncological efficacy. In addition to this, there is a myriad of circumstances, such as those related to ethnic variations and co-morbidities, which uniquely impact on the pharmacological approach in a highly heterogeneous population of castration-resistant prostate cancer (CRPC) patients. Ineffective testosterone suppression through hormonal escape is currently poorly recognized and may result in increased PCa mortality. Until now, the optimal serum testosterone level in patients under castration, and the impact of its variations in patients under LHRH therapy, remain open questions and have been merged to a broad spectra of patients who are highly heterogeneous. This heterogeneity relates to a number of mechanisms regarding response to treatment, which influences the biology of the relapsing tumor and the sensitivity to subsequent therapies in the individual patient. The rationale to achieve testosterone levels below 20–50 ng/dl warrant further investigation as these levels have recently rescued CRPC patients. In the last few years and months, important advancements in prostate cancer treatment have been achieved. Nevertheless, these advances are measured in a few months of additional survival and under high costs, not available to most of the world population, compared with the benefits of hormonal manipulation that are measured in years, there is a huge potential for accessible and durable effect expansion and optimization of treatment, particularly with the current tendency of a more individual approach.

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Introduction

During the last 20 years, surgical castration has been replaced by the medical use of LH-releasing hormone (LHRH) agonists, as a well tolerated treatment option for prostate cancer (PCa), and with comparable testosterone castration cutoff. A level of 50 ng/dl is obtained using old assay methods (Wilke & Utley 1987); however, since 1996, a method using chemiluminescent technology for clinical use (Wheeler *et al*. 1996) resulted in more accurate serum testosterone measurements, and currently the sensitivity of this assay has an accuracy level close to 0.1 ng/dl (Zherdev *et al*. 2003).

In this scenario, the equivalence of surgical and different pharmacological castrations has been contested, and it is of concern that most of the phase III studies for the Food and Drug Administration licensing of LHRH agonists were based on the previous 50 ng/dl castration level defined by the historical assay limitation (Wilke & Utley 1987, Sharifi *et al*. 1996).

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Adding to this controversy is a cluster of evidence supporting the hypothesis 'the lower the better when achieving castration levels of testosterone', based on the data from second-line hormonal manipulation and its molecular basis (Reis 2011), and on better oncological results (survival free of castration-resistant prostate cancer (CRPC)) reported for patients with castration levels <32 ng/dl (Morote *et al*. 2007).

Although an imperative clinical consideration, ineffective testosterone suppression (ITS) through hormonal escape is not recognized when serum testosterone is not systematically monitored, culminating in very scarce data analyzing the impact of serum testosterone levels and breakthrough increases in PCa clinical outcome.

Levels of serum testosterone have been reported to be >20 ng/dl in 13–35% and >50 ng/dl in 2–13% of patients receiving LHRH agonists (Tombal 2005).

Clinical trials comparing outcomes among the various forms of hormonal therapy have reported no survival advantage for orchiectomy that achieves lower testosterone levels than the monthly LHRH agonist (i.e. 3.6 mg goserelin acetate, 3.75 mg leuprolide acetate, or 7.5 mg leuprolide acetate). However, they must be interpreted cautiously once they are not powered to detect small differences in outcome, owing to a relatively short median follow-up (<15 months), and patients' heterogeneity (Kaisary *et al*. 1991, Vogelzang *et al*. 1995).

The fact that values up to 30 ng/dl were also observed in surgically castrated cases (Oefelein *et al*. 2000), and that a modest overall and cancer-specific survival has been shown on meta-analysis for maximal androgen blockade (MAB), obtained by the addition of an oral antiandrogen compared with castration alone (Schmitt *et al*. 2000), supports the results of clinical relevance of 32 ng/dl for an effective castration (Morote *et al*. 2007).

Furthermore, recent data suggest that about 70% of patients experienced decreased prostate specific antigen (PSA) after the LHRH switch, and this decrease appeared more pronounced when switching from leuprolide to goserelin rather than *vice versa* (Lawrentschuk *et al*. 2011). These data suggest that the pharmacodynamics of these agents may be different.

Although CRPC is a more precise and recommended definition compared with androgen independently (Reis 2011), it aggregates a highly heterogeneous population, considering that some trials have shown that individual LHRH analogs can act differently and at their respective doses induce castration over differing time frames and to different levels (Heyns *et al*. 2003).

The degree of potency of LHRH analog peptides varies according to their modified amino acid structures, and thus the effect on the pituitary–gonadal axis may vary with the agent. Also, the method of delivery has an impact on bioavailability and potentially causes differences in androgen levels (Chodak 1989).

In this scenario, ITS may result in increased PCa mortality, and until there is better understanding of LHRH therapy, periodic testosterone monitoring ensures appropriate androgen deprivation. Future studies are warranted to label and classify LHRH agonists from a regulatory perspective in different classes according to their efficacy now there is rapidly growing evidence that the depth of the testosterone nadir is associated with a survival advantage in men with metastatic PCa (Morote *et al*. 2007, Perachino & Cavalli 2008, Perachino *et al*. 2010).

In general, maximal Leydig cell suppression is achieved within the first month after initiation of the 1- or 3-monthly depot preparations of LHRH analogs. An open-label study designed for regulatory authorities to evaluate leuprolide acetate (3.75 mg) depot formulation for commercialization has shown that by day 28, 96.8% (151/156) and 73.1% (114/156) of evaluable patients had testosterone concentrations ≤ 50 and ≤ 20 ng/dl respectively (Marberger *et al*. 2010).

Previous reports on failure to achieve castration have involved leuprolide acetate. Analyses have indicated that inter-patient variance is greater for patients receiving leuprolide (Smith & McGovern 2001, Yri *et al*. 2006).

A cross-sectional, retrospective, non-randomized study showed that 4 out of 65 patients treated with LHRH analog did not have a serum testosterone level within the female range (2.8 nmol/l, 81 ng/dl) during treatment with leuprolide acetate but were within the range with goserelin acetate. This suggests that the LHRH equivalence is valid at the group level, but may not be true for individual patients (Yri *et al*. 2006).

Another study, randomizing 22 patients to receive either goserelin 3.6 mg ($n=11$) or leuprolide 3.75 mg ($n=11$), found that after 28 days, total testosterone decreased to within a range observed following castration (<50.0 ng/dl) in all patients except for one in the leuprolide group. However, the mean rate of decrease in LH concentration was significantly greater in the goserelin group compared with the leuprolide group, and at day 21, compared with goserelin, leuprolide was associated with a significantly smaller dispersion in the rate of decrease in PSA, and the dispersion of the rate of change in total testosterone

concentration was significantly greater with leuprolide than with goserelin at day 21 ($P=0.0235$; folded F test). Furthermore, although the magnitude of the initial LH surge and low incidence of tumor-flare reactions were similar in both groups, leuprolide appears to be associated with a greater initial testosterone surge than goserelin (Tanaka *et al*. 2007).

Although the clinical significance of this difference in testosterone is uncertain, goserelin is the only LHRH agonist shown to improve overall survival when used as an adjuvant to radiotherapy in locally advanced disease (Bolla *et al*. 2002, Pilepich *et al*. 2005) and to radical prostatectomy in patients with node-positive disease (Messing *et al*. 1999).

From 73 consecutive patients with PCa, treated with 3-monthly depot LHRH agonists, the rate of breakthrough increases in serum testosterone over any castrate level over time was considerable. Irrespective of the LHRH agonist, the probability of a future breakthrough increase could be predicted, and three determinations of <20 ng/dl should guarantee no future breakthrough increases of >50 ng/dl (Morote *et al*. 2009).

A meta-analysis including 12 trials, comparing LHRH agonist monotherapy with orchiectomy or diethylstilbestrol, suggested that they are essentially equivalent to orchiectomy in terms of survival; however, none of these trials directly compared the LHRH agonists (Seidenfeld *et al*. 2000) and the power of the study might be regarded as insufficient to detect very small survival differences.

The reason for insufficient androgen deprivation during LHRH treatment is not understood, and there is speculation over whether obesity interferes in the pharmacokinetics of the drugs used to alter the LHRH receptor, antibodies against the drug, blocked absorption, or its rapid metabolization.

It was shown that despite lower pretreatment serum testosterone levels, obese men (body mass index (BMI) >30 kg/m^2) had total and free testosterone levels 1.8 and 2.3 times greater, respectively, than those in normal men after 48 weeks of leuprolide 3-month (22.5 mg) depot treatment (Smith 2007).

Also, it was proposed that the effects of LHRH agonist in suppressing serum testosterone levels differ among the races. Although there were testosterone levels just outside the castrate range in a few patients during treatment, 1- and 3-monthly formulations of leuprolide and goserelin had equivalent and sufficient effects to suppress serum testosterone levels in a retrospective study including 232 Japanese men with PCa (Fujii *et al*. 2008).

In the 2001 version of the PCa guidelines, the National Comprehensive Cancer Network (NCCN) recommended that orchiectomy or MAB should be considered if a patient's serum testosterone level was >20 ng/dl during GNRH agonist monotherapy, also known as LHRH agonist (National Comprehensive Cancer Network. Treatment guidelines for patients: prostate cancer. www.nccn.org/index.asp (version II, last update 2001)). This threshold is not evidence based and this was not recommended in the subsequent versions of the NCCN guidelines while better evidence was awaited.

A modest advantage in the 5-year cancer-specific survival probabilities in favor of those patients randomized to MAB (1.8–5%; Prostate Cancer Trialists' Collaborative Group 2000, Schmitt *et al*. 2000) indicates the potential impact of further hormonal manipulation in patients with testosterone levels in the range of 20–50 ng/dl as well as in those patients failing to reach the standard castration level (<50 ng/dl).

Similarly, the sequential responses to hormonal manipulation, known as secondary hormonal therapy, implies that androgen receptor (AR) signaling remains an important therapeutic target and also relies on the fact that CRPC is not necessarily androgen-independent and is susceptible to further hormonal manipulation. Abiraterone, a CYP17 inhibitor that effectively blocks the conversion of androgens from non-gonadal precursors, thus reducing testosterone to undetectable levels, has recently been proved to extend survival rates for men with metastatic CRPC who have progressive disease even after first-line chemotherapy treatment (Reis 2011).

In this context, although better evidence is necessary, the rescue of CRPC patients by using abiraterone supports the rationale of an important role for androgen depletion or androgen levels below 50 ng/dl or even below 20 ng/dl (Reis 2011). The rationale to achieve testosterone levels below 20–50 ng/dl needs to be studied further in the PCa scenario, for those classified as CRPC and even for the hormone-naive patients.

Until now, the optimal serum testosterone level in patients under castration and the impact of its variations in patients under LHRH therapy remain uncertain. In the current scenario of advancements measured in a few months and under high costs, which are not available to most of the world population, compared with the benefits of hormonal manipulation that are measured in years (Reis 2011), this denotes a huge potential for accessible and durable effect expansion and optimization of treatment, particularly for the current tendency of a more individual approach.

Adding to this complex and challenging scenario is the fact recently confirmed by our group that the efficiency of the different available pharmacological castration solutions aimed at reaching castration levels, mainly for cutoff $\leq$20 ng/dl, can vary significantly; surprisingly, about half and one-third of patients did not achieve castration levels of 20 and 50 ng/dl respectively (Silva *et al*. 2012).

On the other hand, the differential response to androgen withdrawal and the resulting effect on survival have motivated important studies supporting the critique concerning the hypothesis of all or none of the manner presenting a response to androgen withdrawal (Hussain *et al*. 2006).

We have previously highlighted (Reis 2011) that although the AR is the major therapeutic target of PCa, there are currently no clinical studies available in which the AR status was considered in the study design. Furthermore, to date, even with better understanding of the molecular pathways behind CRPC, no study on secondary hormonal treatment with strong methodology has shown a benefit in terms of survival, but most trials have been smaller and heavily biased for patients' heterogeneity.

It is of concern that although in the last 2 years, three approved treatments based on Phase III trials have demonstrated modest survival improvements (measured in months) in CRPC (cabazitaxel (potent taxane able to bypass the main resistance mechanism to docetaxel drug efflux pump, *P*-glycoprotein 1; approved in 2010); sipuleucel-T (immunotherapeutic agent through dendritic cells; approved in 2010); and abiraterone (orally active, potent and irreversible inhibitor of CYP17 – a critical enzyme in androgen biosynthesis; approved in 2011)), palpable advancement in hormonal management has been scarce and underexplored (Reis 2011, Silva *et al*. 2012).

Recently, stunted attempts by more complete and occasionally intermittent androgen blockade have been described (Scholz *et al*. 2007); however, these strategies are clearly less appetizing to the pharmaceutical industry, the well-recognized engine of research funding in the field of clinically advanced PCa treatment.

The very first and essential steps might be to know the real level of ITS through hormonal escape, which can vary individually among patients, and its tangible rate as they are currently poorly recognized and have been merged to patients' heterogeneity. Aspects such as ethnic and co-morbidity variations are fundamental in this setting, and therefore studies around the world including different races and different patients' profiles would be beneficial.

Even through relatively methodologically unsound studies, a broad spectra of patients has been identified, involving a number of mechanisms regarding the response to treatment, and many prognosticators, including: no prior hormone exposure, responsiveness to second-line therapy, and the effects of a drug on each parameter of disease independently (Scher *et al*. 1997, Suzuki *et al*. 2008).

The specific hormone therapy administered and the response to that therapy can influence the biology of the relapsing tumor and the sensitivity to subsequent therapies, and are therefore predictors of increased survival in individual patients. In this regard, a PSA of 4 ng/ml or less after 7 months of androgen deprivation through goserelin and bicalutamide is a strong predictor of survival (Hussain *et al*. 2006).

There are unquestionably different molecular determinants on why one cancer would respond to possibly slightly higher levels than another, and this presumably relates to how the AR is working (Scher *et al*. 1997). Briefly, at least five fundamental mechanisms are AR mediated (three of which depend on ligand signaling), which contribute to the stem-cell pathway: persistence of intratumoral androgens as a result of *in situ* steroidogenesis or adrenal source; *AR* mutations that allow promiscuous activation by the otherwise nonsignaling ligands; wild-type *AR* gene amplification; alterations in the AR co-activator:co-repressor ratio that impact transcription; and outlaw AR pathways that bypass the need for androgens by signaling through cross talk with other ligand-bound receptors, cytokines, or transactivation of activated tyrosine kinase receptors in the cytosol (consider reference (Reis (2011)) for a comprehensive review on the molecular basis).

Further research is required to gain greater understanding of the issues, particularly with regard to the evidence-based clinical aspects. Therefore, appropriately large cohorts of patients with the right design, precise targets, and very long follow-up periods, combined with oncological and survival primary outcomes, are necessary to avoid future studies being underpowered (Reis *et al*. 2010).

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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