



Original Article

Intraprostatic hormone dosage: Validation of a novel prostate biopsy technique



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ABSTRACT

Background: Advances in chromatography and mass spectrometry have allowed us to develop a novel technique for measuring intraprostatic hormone concentrations directly on prostate needle biopsies, rather than using traditional punch excision. This has significant clinical implications as intraprostatic dihydrotestosterone and testosterone levels could help monitor prostate growth, neoplasia and castration resistance.

Methods: Patients undergoing radical cystoprostatectomy for bladder cancer were prospectively included. Each prostate specimen received one 90 mg punch excision and six needle biopsies. Intraprostatic hormones were dosed through gas chromatography-mass spectrometry.

Results: We included twenty patients, of which eleven were incidentally diagnosed with prostate cancer; four had ISUP 1 (20%) and seven had ISUP 2 (35%). The prostate biopsy technique was unable to obtain measures for testosterone, Delta-4-androsterone and androstenedione. Tissue concentrations of DHEA, DHT, E1 and E2 can be obtained with no significant difference from the reference established on a punch from a single biopsy core sample.

Conclusions: Our study demonstrates that intraprostatic concentrations of DHEA, DHT, E1 and E2 can be measured without significant difference from the reference established on a single punch excision. This finding opens the way to research on the interactions between endocrinology and prostate oncogenesis and particularly on the mechanisms of resistance to hormone therapies *in vivo*.

Level of evidence: 2

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1. Introduction

Huggins and Hodges (1941) first exposed the central role of androgen signaling in prostate cancer eighty years ago by showing that orchiectomy induces considerable tumor regression. Their discovery was recognized with the Nobel Prize in Medicine in 1966, and since then, it has paved the way of therapy for advanced diseases [1]. Later on, 5alpha-dihydrotestosterone (DHT) was found to be the major androgen found within the prostatic cell nucleus with a tenfold potency to stimulate androgen-receptor genes compared to testosterone (T) [2]. A possible link between elevated levels of 5alpha-Dihydrotestosterone (DHT) and prostate cancer (pCa) has been proposed. This association could be attributed to the induction of intraepithelial neoplasia [3] or elevated cellular

proliferation [4], particularly through TMPRSS2-ERG fusions, which are present in up to 50% of pCa cases [5].

Despite the undeniable interest in their measurement in this context, the assessment of intraprostatic steroid concentrations is poorly used. The accuracy of intraprostatic steroid determination is contingent upon the method and area of sample extraction as well as the process leading up to end-point determination. The use of punch biopsy on prostate tissue has been customary for many years, yet a more modern approach, prostate needle biopsy, is becoming the new standard for intraprostatic hormone dosing [6]. This shift is made possible by advances in analytical techniques such as the combination of chromatographic methods with mass spectrometry (MS), which enables the use of less than 5 mg of tissue [7].

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A recent review has not demonstrated any significant difference in intraprostatic androgen levels, particularly DHT, between normal adult prostate tissue, benign prostatic hyperplasia (BPH) tissue and prostate cancer tissue, nor within the histologically distinct regions of the prostate [6]. However, this review is comprised of studies with substantial methodological differences. The STERPROSER trial [8] found that high volume prostates had a higher concentration of dihydrotestosterone (DHT), while the STERKPROSER trial [9] found that prostate cancer (pCa) tissue had a fourfold decrease in testosterone (T) concentration compared to normal prostate tissue, particularly in the peripheral zone.

The primary objective of this study was to compare the intraprostatic concentrations of sex steroids in punch samples (reference technique) to an increasing number (1 to 3 cores) of prostate needle biopsies (experimental technique) on fresh surgical specimens obtained after radical cystoprostatectomy.

2. Methods

2.1. Study design

This prospective, non-interventional study was conducted at a French urological academic hospital between June 2020 and July 2021. Inclusion criteria for the study were: adult males (18+ years) undergoing a radical cystoprostatectomy for bladder cancer. Written informed consent was obtained from all participants. Exclusion criteria included a history of first- or second-generation androgen deprivation therapy (ADT), any taxane-based chemotherapy or corticosteroid treatments, inability to speak French, being deprived of liberty or under guardianship.

2.2. Sample collection

The radical cystoprostatectomy has been performed as part of the usual care within a maximum of 7 days after the inclusion visit.

In the operating room and following the radical cystoprostatectomy, the prostate has been sent to the Department of Pathology of the Foch Hospital according to the pathway of samples for extemporaneous examination in order to reduce the delay.

A punch of 90 mg of prostate tissue and 6 cores of 30 mg each by single-use 18 Gauge needle biopsy were collected by a single pathologist from the surgical specimen upon its arrival and the fragments were frozen extemporaneously.

The surgical specimen was then taken to the Department of Pathology for standard histological study following the guidelines of good clinical practice.

2.3. Measurement of steroid levels in prostate tissue by gas chromatography-mass spectrometry (GC-MS)

The six frozen cores and the punch will be sent secondarily to the INSERM 03-37 laboratory, where tissue assays will be performed by mass spectrometry with gas chromatography.

The following hormones were measured in homogenized prostate tissue: testosterone (T), dihydrotestosterone (DHT), Delta-4-androstenedione (4-dione), androsterone, Delta-5-androstenediol (5-diol), dihydroepiandrosterone (DHEA), estrone (E1), estradiol (E2), conjugated steroids (DHEA Sulfate, Estrone Sulfate, Testo Glucuronide, DHT glucuronide).

The procedure (GC-MS) for the determination of prostate tissue steroid level was similar to that described previously [8,9]. The steroids were derivatized with pentafluorobenzylhydroxylamine (P4192 Aldrich). The molecular mass of the derivatized steroids, target ions analyte/internal standard were testosterone 482/485, DHT 484/487, DHEA 482/485, DHEA-S482/488, estrone 464/468, estrone sulfate 464/468, estradiol 660/664, androstenediol 678/683.

2.4. Statistical analysis

The assays being quantitative, the agreement between the assay on biopsy fragments and the punch was evaluated by the calculation of a correlation coefficient, with visual control of the “cloud of points” allowing the qualitative distinction of outliers and the verification of the linearity of the relationship. The Spearman correlation coefficient, which consists in calculating the correlation, not between the values taken by the variables, but between the ranks of these values after having ranked them from the lowest to the highest, was preferred to the Pearson correlation coefficient. It is less sensitive to outliers and deviations from the assumption of normality of the distributions than the Pearson correlation coefficient.

However, the Pearson correlation coefficient was also calculated to ensure that the results of the two indicators were consistent.

A comparison of the mean values of the punch and biopsy fragment assays was also performed to ensure that the assays are not only correlated but also close in terms of mean values.

The Wilcoxon paired-data test was used to detect a statistically significant difference between the mean values of the assays: the punch technique is compared to one, two and three biopsies in Pvs1, Pvs2 and Pvs3, respectively.

These calculations and the visualization of the scatter plot were used to define the optimal number of biopsy cores to use to have significantly comparable assay results with the punch, by analyzing the gain in correlation observed by going from one biopsy core to two and then to three. If even with 3 fragments, the Spearman correlation coefficient was less than 0.70 for one assay, it was concluded that this biopsy assay was not reliable.

By including 20 subjects, an observed Spearman correlation coefficient greater than 0.38 will indicate that there is a significant relationship at the 5% risk between the punch and biopsy assays.

Thus, 20 subjects will allow detection with sufficient power and a correlation higher than 0.38.

3. Results

Twenty patients were included, the mean age was 65.5 years with an average BMI of 25 (Table 1). More than half ($n = 11$) of patients presented an incidentally diagnosed pCa: four ISUP 1 (20%) and seven ISUP 2 (35%). Intraprostatic hormone concentrations are presented in Table 2.

No differences were found between Punch excision and one, two, or three needle biopsy cores for DHEA-S, E1, or E2. Regarding DHT, no significant difference was found between the mean concentration values obtained from the punch excision and one or two needle biopsy cores; however, 3 needle biopsy cores had a significantly lower mean than the Punch excision: 5.58 ng/mL [4.98–6.93] vs. 7.14 ng/mL [6.27–8.40], $P = 0.02$. As a corollary, tissue concentrations of DHEA, DHT, E1, and E2 can be obtained with no significant difference between the reference (punch excision) and a single needle biopsy core. Punch excision DHEA concentrations were significantly lower than with one and two biopsy cores: 21.75 ng/mL [16.10–41.70] vs. 25.92 ng/mL [19.09–45.33], $P = 0.04$ and 27.06 ng/mL [19.73–36.67], $P = 0.003$, respectively. The prostate needle biopsy technique was unable to obtain measures for testosterone, Delta-4-androstenedione, and androstenedione.

Table 1
Population characteristics ($n = 20$).

	$n = 20$
Age, year	66 [60–76]
Body mass index, kg/m ²	25.1 [22.7–25.9]
Gleason score	7 [6.7] $n = 11$
ISUP classification	
Group 1	4 (20)
Group 2	7 (35)

Results are presented as number (percentage) or median [25–75th percentiles].

Table 2Hormone concentrations (ng/mL) per group, $n = 20$ except when specified.

	Punch	1 core	2 cores	3 cores	P-value
Testosterone (T)	0.10 [0.09–0.13] $n = 5$				NA
Dihydro-testosterone (DHT)	7.14 [6.27–8.40]	7.38 [6.90–8.32]	7.81 [6.63–8.54]	5.58 [4.98–6.93]	Pvs1: 0.23 Pvs2: 0.22 Pvs3: 0.02
Delta-4-androstenedione (D4)	0.18 [0.14–0.26] $n = 18$		1.25 $n = 1$	0.04 $n = 1$	NA
Androsterone (ADT)	0.22 [0.11–0.26] $n = 13$			0.25 $n = 1$	NA
Delta-5-androstenediol	1.21 [0.76–2.08]	3.1 $n = 1$	2.83 [2.52–3.14] $n = 2$	2.53 [2.04–3.37] $n = 9$	Pvs3: 0.15
Dehydroepiandrosterone (DHEA)	21.75 [16.10–41.70]	25.92 [19.09–45.33] $n = 19$	27.06 [19.73–36.67]	26.46 [16.41–34.29]	Pvs1: 0.04 Pvs2: 0.003 Pvs3: 0.30
Estrone (E1)	0.12 [0.07–0.19]	0.26 [0.23–0.29] $n = 5$	0.21 [0.18–0.22] $n = 9$	0.17 [0.14–0.21] $n = 12$	Pvs1: 0.81 Pvs2: 0.07 Pvs3: 0.98
Estradiol (E2)	0.03 [0.02–0.04]	0.04 [0.04–0.05] $n = 3$	0.04 [0.04–0.05] $n = 4$	0.03 [0.02–0.04] $n = 15$	Pvs1: 0.75 Pvs2: 0.12 Pvs3: 0.84
DHEA Sulfate	186.2 [124.4–250.1]	551.6 [395.6–707.6] $n = 2$	389.6 [225.7–392.4] $n = 5$	266.2 [242.8–435.6] $n = 10$	Pvs1: 0.50 Pvs2: 1.00 Pvs3: 0.43
Estrone sulfate	0.82 $n = 1$	13.92 [11.75–16.09] $n = 2$	7.18 $n = 1$	255.27] $n = 1$	NA

4. Discussion

We showed that DHT concentrations were comparable between the prostate punch technique and one or two biopsies and could be an easily accessible surrogate for intraprostatic DHT concentration. This is an important finding as it shows the feasibility of *in vivo* prostate biopsies to measure prostatic hormones. Intratumoral synthesis of dihydrotestosterone (DHT) is of utmost importance as it could partly explain the castration resistance of prostate cancer [10]. Intraprostatic DHT is associated with cancer aggressiveness: its concentration is lower in high Gleason stages [11]. Interestingly, serum and intraprostatic hormonal concentrations do not react the same way to medical castration: a study on thirteen healthy men found a decrease in serum T by 94% whereas prostatic T and DHT levels were 70 and 80% lower [12], further advocating the importance of *in vivo* intraprostatic dosage. However, three biopsies and one or two biopsies for DHT and DHEA respectively differed from the punch concentration, this could be explained by concentration heterogeneity within the prostate. DHEA can be a direct ligand for the AR and induce weak androgenic effects, potentially promoting prostate cancer growth [13].

The prostate biopsy technique was unable to obtain measures for testosterone, Delta-4-androstenedione, and androstenedione. Conversely, these hormones have less value than intraprostatic DHT: while T concentrations in recurrent PCa and androgen-stimulated benign prostate remain stable, DHT prostatic levels decrease by 91% in recurrent PCa [14]. This suggests that recurrent PCa prostate may develop the capacity to biosynthesize testicular androgens from adrenal androgens or cholesterol, thus explaining the efficacy of abiraterone in castration-resistant prostate cancers. Dosing intraprostatic hormones could have clinical implications to monitor the efficacy of second-generation anti-androgens. The determination of steroid concentrations is questionable when performed by immuno-histochemical methods [15], thus alternatives using the GC-MS technique should be used to dose these hormones.

Steroids level degradation occurs between sampling and freezing, once frozen, they no longer present a risk of degradation [16], all of the samples in this study were frozen within less than an hour while assays were performed within one month after freezing.

The proportion of incidentally diagnosed pCa in patients undergoing radical cystoprostatectomy (CPT) for bladder cancer was higher than the 28% reported in literature [17]: this can partly be explained by the centralized pathological examination.

The present study presents several limitations: first, it lacks information on the localization of the prostate biopsies: it has been shown that T and DHT accumulate in the stroma of enlarged prostates and the degree of accumulation correlated with prostate volume [18,19]: attributed to either higher 5 α -reductase expression or the lower expression of downstream metabolizing enzymes [20]. This study suggests the realization of three systematic prostate biopsies. We chose not to correlate intraprostatic and circulating hormonal concentration because circulating concentrations of sex steroid hormones have proven to be poor surrogate measures of the intraprostatic hormonal milieu [21]. Conjugated excretion product dosage was not included in our protocol.

The strengths of this study are the use of the GC/MS method, which has proven to be the most reliable [7] in accordance with current guidelines [15] and a relatively large number of patients. This study is the first prospective comparative study of these two techniques: previous comparisons between punch and biopsy were only done in retrospective meta-analyses [6]. Biopsies allow a standardization of the intraprostatic tissue sampling and therefore dosage technique. Differentiation of peripheral and transitional zones is easier on these biopsies.

5. Conclusion

Using the reference technique based on GC-MS, the evaluation of intraprostatic concentrations of DHEA, DHT, E1, and E2 could be obtained without significant differences from the reference established on a single biopsy core. The demonstration of the possibility of a reliable determination of these hormones within prostate tissue opens the way to research on the interactions between endocrinology and prostate oncogenesis and particularly on the mechanisms of resistance to hormone therapies.

Disclosure of interest

The authors declare that they have no competing interest.

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