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Sex steroids in serum and prostatic tissue of human cancerous prostate (STERKPROSER trial)

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Background: Currently, there is no consensus regarding the expected concentration levels of intra-prostatic sex steroids in patients with Prostate Cancer (PCa). Our objective was to assess the concentration levels of sex steroids in prostatic tissue and serum, in two cohorts of patients with localized PCa or benign prostatic hyperplasia (BPH).

Methods: Between September 2014 and January 2017, men selected for radical cystectomy (for bladder cancer) or open prostatectomy (for BPH), and men selected for radical prostatectomy for localized PCa were included. Blood samples were collected at baseline before surgery, and steroid concentrations were assessed following the recommendations of the Endocrine Society. Intra-prostatic samples were collected from fresh surgical samples, and assessed by gas chromatography and mass spectrometry (GC/MS). Permanova analysis was performed. Analyses were adjusted for age, prostate weight, and prostate-specific antigen (PSA) level.

Results: A total of 73 patients (41 patients with PCa and 32 patients with BPH) were included in this study. Patients with PCa were younger, and had smaller prostate volumes with higher levels of PSA. The levels of Total Testosterone (TT), Di-Hydro-Testosterone (DHT), and Estradiol (E2) in the serum were not significantly different between PCa and BPH. In PCa tissue, TT concentrations were significantly lower (0.11 ng/g vs 0.47 ng/g, $P = 0.0002$), however its derivative E2 had significantly higher concentrations (31.0 ng/g vs 22.3 ng/g, $P = 0.01$). DHT tissue concentrations were not significantly different between the two groups (5.55 ng/g vs 5.42 ng/g, $P = 0.70$). Intra-prostatic TT concentrations were significantly lower in the peripheral zone than in the central zone for the CaP group (0.07 ng/g vs 0.15 ng/g, $P = 0.004$).

Conclusions: Patients with PCa had lower intra-prostatic TT and higher E2 concentrations levels compared to the patients with BPH. PCa seem to consume more TT and produce more E2, especially in the peripheral zone.

KEYWORDS
5-alpha-reductase, aromatase, prostate cancer, sex steroids, total testosterone

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1 | INTRODUCTION

Androgens are involved in the development and evolution of both prostate cancer (PCa) and benign prostate hyperplasia (BPH).[1] Testosterone is the main androgen in the blood, while dihydrotestosterone (DHT), its active metabolite, is mainly present in the prostate gland.[1]

To date, the impact of each sex steroid in the PCa carcinogenesis remains unclear. As prostatic levels of androgens have endocrine and intracrine sources, intra-prostatic concentration levels of sex steroids might not correlate with serum levels.[2] The combined assessment of serum and intra-prostatic concentration levels of sex steroids, performed simultaneously, may provide new pathophysiological assumptions.

Currently, there is no consensus on the expected concentration levels of intra-prostatic sex steroids in patients with PCa. Several studies have been based on tissue concentration levels of sex steroids in men with PCa with conflicting results,[3] using improper methods, as most of these trials were done in the immuno-assay era[4–8] and/or showing partial data.[9,10] Given that gas chromatography—mass spectrometry (GC/MS) confers greater sensitivity and specificity to sex steroid measurements, this method is now mandatory according to the Endocrine Society guidelines.[11] In the largest review of the literature, van der Sluis[3] emphasizes these facts and calls for strong data obtained through strict methodology. Accordingly, we recently published our data regarding BPH.[12] We now move forward, using the same protocol, in studying data regarding localized PCa.

Using GC/MS, Olsson et al[13] compared various androgens (Testosterone, DHT, Androsterone) in serum samples drawn from peripheral blood vein and prostatic vein, in patients who underwent radical prostatectomy for PCa. The authors showed a significant positive correlation between the local prostatic serum DHT levels and circulatory serum levels. However, correlation between sex steroid concentration levels in prostate tissue and prostatic vein has not been assessed. Arguably, PCa can independently synthesize DHT.[14] Therefore, sex steroid tissue concentration comparisons between PCa and BPH is an important issue. In addition, because PCa is more likely to be located in the peripheral zone, evaluating intra-prostatic sex steroid levels according to the prostate area (ie, peripheral or central) may improve our understanding of PCa development.

Hence, our main objective was to assess and to compare the concentration levels of serum and prostate tissue sex steroids in two cohorts of patients with PCa or BPH.

2 | PATIENTS

Between September 2014 and January 2017, men referred to our Department of Urology have been screened as follows:

1. for BPH, men selected for radical cystectomy (due to bladder cancer) or open prostatectomy (for BPH) were screened for inclusion in the STERPROSER trial (NCT02778243)
2. for PCa, men selected for radical prostatectomy for localized PCa (Stage I or II) were screened for inclusion in the ANDROCAN trial (NCT02235142).

Common exclusion criteria were: prior PCa local therapy (ie, irradiation or focalized ultrasound); hormonal treatment (ie, luteinising-hormone-releasing-hormoneagonist or antagonist, antiandrogens), and any treatment that could interfere with hormone levels (ie, Prednisone, Ketoconazole, Abiraterone acetate, Bicalutamide or 5-alpha-reductase inhibitors ongoing, or stopped for less than 3 months before the intervention). Written informed consents were obtained from all the patients. Clinical data including age, size, weight, and waist circumference were prospectively collected. Fat mass was assessed using impedance analysis of body composition (Inner Scan™ Body Composition Monitor BC-543, Tanita, Arlington Heights, IL) and biological data.

3 | METHODS

3.1 | Collection of blood samples for the assessment of basal levels of sex steroids

Blood samples (30 mL) for steroid concentration determination were collected before surgery, after an overnight fast, between 7 and 10 a.m., as recommended in the Endocrine Society guidelines.[15] All samples were stored immediately at −20°C.

3.2 | Collection and preparation of prostate tissue for the assessment of intra-prostatic levels of sex steroids

The samples were taken extemporaneously. The fresh prostate were weighed and sliced into 5 mm thick slices. Ninety milligrams samples were taken with a punch of 8 mm diameter from both peripheral and central zone of the prostate. The prostate samples were separately weighed then placed in liquid nitrogen to be transferred in freezers at −80°C.

Each frozen sample of prostate was cut into thin slices and the hashed tissue was wrapped between two aluminum foils, folded over to form a papillote, immediately plunged into liquid nitrogen and pulverized by two hammer blows. The resulting prostate tissue powder was then pooled and mixed homogeneously at 4°C. Premixed homogeneous prostate tissue (200 ± 50 mg) were introduced and weighed in a Lysing Matrix D tube (MP Biomedicals, Illkirch-Graffenstaden, France), 50 µL deuterated methanol internal standard working solution and 1 mL of cold saline water were added. The sample was homogenized.

After the grinding cycles, each sample was transferred to a glass tube for fast cooling in crushed ice. The tissue residuals in the Lysing Matrix D tube were recovered with one supplemental mL of cold saline water and two times 1.5 mL of pure methanol (HPLC grade), added to the glass tube, vortexed for 2 min and centrifuged for 30 min at 4°C (1700 g). The methanol/OH2 supernatant containing

free and conjugated steroids was collected and evaporated until complete removal of methanol.

The remaining aqueous phase was completed (if necessary) to 2 mL and purified on C18 500 mg Hypersep mini-columns (Thermo Fisher, Courtaboeuf, France). The un-conjugated (or free) steroid (fraction A) were eluted by ethyl acetate (4 mL) and evaporated, then the conjugated steroids (fraction B) were eluted by methanol (4 mL) and evaporated.

For solvolysis, the dry residue of the conjugated steroid fraction B was re-dissolved in physiological serum, and sulphuric acid 2N, 100 µL + ethyl acetate 3 mL was added, and kept at 37°C during 12 h. Then, ethyl acetate was added to complete the initial volume. After centrifugation, the organic upper phase was collected in a new glass tube, evaporated to dryness, and neutralized with 1 mL of NaHCO3.

While the prostate samples were weighed, a "mirror" box for each sample was prepared; a thin slide colored by HES served to check the presence or absence of cancer, abundance of glandular stroma or glands, and the quantity of tumor tissue. PCa was defined by the presence of prostatic adenocarcinoma as recommended by the International Society of Urologial Pathology.[16] Samples from specimens with other malignancy than prostatic adenocarcinoma were excluded. For BPH, samples from specimens with evidence of any malignancy were excluded.

3.3 | Steroid assay

Steroid concentrations were measured at a central laboratory using gas chromatography and mass spectrometry (GC/MS).[17,18] The analyzed compounds in serum were Total Testosterone (TT), DHT, Estrone (E1), Estradiol (E2), Dehydroepiandrosterone (DHEA), Δ5-androstenediol (Δ5), and Dehydroepiandrosterone sulfate (DHEA-S). Briefly, TT, DHT, DHEA, de-sulfated DHEA-S, E1, de-sulfated E1-S, E2, and Δ5 were derivatized with pentafluorobenzoyl chloride (103772-1G, Sigma–Aldrich, Darmstadt, Germany). Final extracts were reconstituted in iso-octane, then transferred into conical vials for injection into the GC system (6890N, Agilent Technologies) using a 50% phenylmethyl-polysiloxane VF-17MS capillary column (20 m × 0.15 mm, internal diameter, 0.15 µm film thickness) (Varian, France). An HP5973 (Agilent Technologies) quadrupole mass spectrometer equipped with a chemical ionization source and operating in single-ion monitoring (SIM) mode was used for detection and measurement. DHEA-S and E1-S were measured as DHEA and E1. The deuterated internal standards used were the followings: T-d3, DHT-d3, DHEA-d3, DHEA-S-d6, E1-d4, E1-S-d4, E2-d4, and 5α-androstane-3β17β-diol-d3 (3β-diol-d3; corresponding deuterated internal control of 5-diol). All were obtained from C.D.N. Isotopes (Cluzeau Info Lab, Sainte Foy La Grande, France).

For the measurements in the prostate, the dried un-conjugated steroid *fraction (A)* and dried de-conjugated steroid (*fraction B)* were re-dissolved in 1-chlorobutane (3 mL). This organic phase was collected and purified on conditioned LC-Si SPE columns. The columns and adsorbed material were washed with ethyl acetate /hexane (6 mL; 1/9, v/v). Un-conjugated and de-conjugated steroids were eluted using ethyl acetate/hexane (4 mL; 1/1, v/v), then evaporated at 60° and re-dissolved in a mixture of charcoal dextran stripped fetal bovine serum. The limit of detection or LOD was determined by measuring the signal to noise (s/n) ratio of the standards, a s/n of three was the minimally accepted value. The limit of quantification or LOQ for each steroid was determined as a statistical measure from repeated measurements of the standard curves with less than 20% variability.[19] The LOQs and LODs are reported in supplementary Table S1.

The procedure (GC/MS) for the determination of prostate tissue steroid level was similar to that described previously. 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. The ion chromatogram showing the separation of the analytes was presented in Figure 1.

The recovery was carried out by overloading a pool (150 mg) of pulverized homogenous BPH tissue by a methanolic mixture (of the eight assayed steroids + a methanolic mixture of the corresponding deuterated steroids). The recovery for each steroid was between 94.4% and 109.1%. The recovery being close to 100% we may ascertain that the investigated prostatic tissue did not hamper, that is, increase or decrease the mass spectrometry signal and consequently that no matrix effect can be exhibited.[20]

Bioavailable testosterone (BT) was calculated with a standardized formula using specific association constants (optimization of Ka and Ks of TT for SHBG according to Giton et al[21]) and for albumin.[21–23] Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Sex Hormone-Binding Globulin (SHBG) were assessed by radio-immunoassay.

3.4 | Statistical analysis

Study was designed with two arms: PCa or BPH. Calculations were done using NCSS, version 11 (NCSS, Kaysville) and Primer, version 7 (Quest Research, Auckland, NZ).

To accommodate for non-normal distributions, summaries were obtained using bootstrap and Hodges-Lehman estimators. Simple group comparisons were done using a randomization *t*-test with 10 000 replications.

Since the study was designed with two arms (PCa or BPH), and two zones in each prostate (central and peripheral; total prostatic tissue concentrations were calculated by the mean between the concentrations of the central zone and the peripheral zone), Permanova[24] a procedure analogue to ANOVA based on permutations was used to obtain probabilities for main factors and their interactions.

4 | RESULTS

A total of 73 patients (41 patients with PCa and 32 patients with BPH) were included in this study. Clinical and morphological characteristics of the population are presented in Table 1. Patients with BPH had an increased prostate volume and a tendency to be older. PSA level was

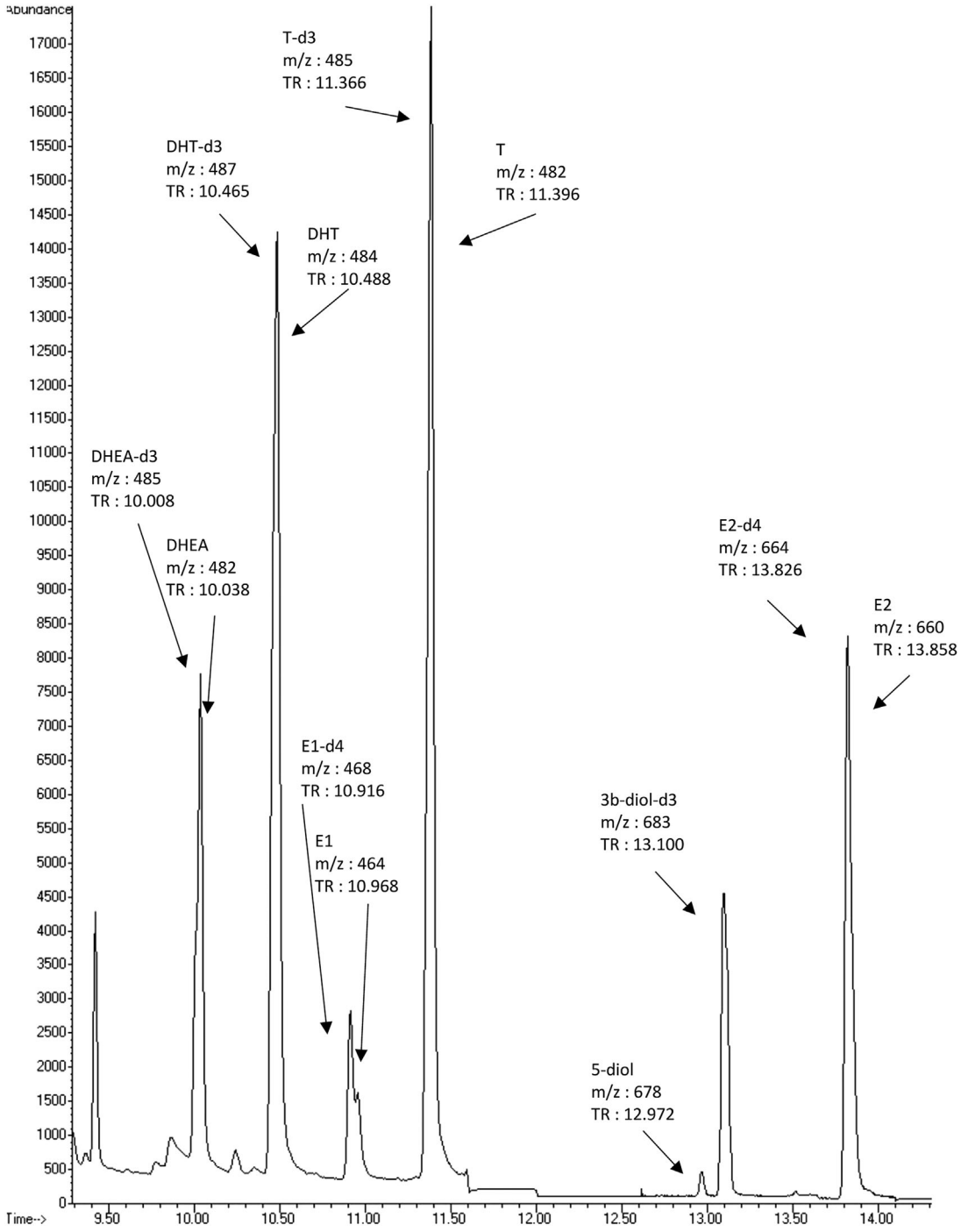

FIGURE 1 The ion chromatogram showing the separation of the analytes

significantly higher in the PCa group compared to the BPH group (9.2 ng/mL, 95%CI [7.4–10.9 ng/mL] vs 5.1 ng/mL, 95%CI [2.8–7.3 ng/mL], $P = 0.003$). Thus, subsequent analyses were adjusted on age, prostate weight, and PSA.

Serum testosterone levels were not significantly different according to the presence or absence of PCa. Similar findings were observed for TT, BT, and androgen-binding protein, SHBG (Table 2). In addition, concentration levels of testosterone adrenal precursors (DHEA, DHEA-S, and Δ5) were not significantly different, as well as their active metabolite (DHT) or their derivative E2. However, E1 serum level was significantly lower in the PCa group. Pituitary hormones (FSH and LH) serum concentration levels were not significantly different between groups.

In total prostate tissue (Table 2), TT concentrations were significantly lower in the PCa group (0.11 ng/g, 95%CI [0.05–0.18 ng/g] vs 0.47 ng/g 95%CI [0.28–0.66 ng/g], $P = 0.0002$). Accordingly, its direct derivative, E2 had significantly higher concentration levels in the PCa group (31.0 ng/g, 95%CI [27.6–34.4 ng/g] vs 22.3 ng/g 95%CI [16.3–28.4 ng/g], $P = 0.01$). DHT tissue concentrations were not significantly different between the

TABLE 1 Comparison of patients' features according to the prostate malignancy

	PCa (mean 95%CI)	BPH (mean 95%CI)	*P*
Age (years)	64 (61–66)	67 (64–69)	0.08
Height (cm)	174.9 (172.5–177.2)	174.2 (171.8–176.7)	0.72
Body weight (kg)	79.9 (75.1–84.7)	78.9 (74.7–83.1)	0.77
BMI (kg/m^2)	26.0 (24.8–27.2)	26.0 (24.7–27.2)	0.95
Fat mass (%)	25.4 (23.1–27.7)[a]	24.9 (22.4–27.4)	0.78
Waist circumference (cm)	99.9 (95.9–104.0)[b]	99.8 (95.8–103.7)[b]	0.96
Prostate Weight (g)	40.5 (34.5–46.1)	57.5 (45.4–69.7)	0.011
PSA (ng/mL)	9.2 (7.4–10.9)[c]	5.1 (2.8–7.3)	0.003

[a]Only 38 cases in the cancerous prostate group.
[b]Thirty cases in the non-cancerous prostate group, 38 cases in the cancerous prostate group.
[c]Forty cases in the cancerous prostate group.

two groups (5.55 ng/g [5.1–6.0 ng/g] vs 5.42 ng/g 95%CI [4.88–5.96 ng/g], *P* = 0.70). Above the other androgens tissue concentration levels, there were no significant differences, either for precursors (DHEA, DHEA-S, and Δ5) nor for derivatives (E1).

Regarding the different areas of prostatic tissue (Table 3), the same differences were observed: TT was significantly lower in the PCa group, both in the central zone and the peripheral zone. E2 tissue concentration levels were likewise superior in the central zone but not in the peripheral zone.

Moreover, after comparing the tissue concentration levels according to the prostate area (Table 4), TT was significantly lower in the peripheral zone compared to the central zone in the PCa group (0.07 ng/mL, 95%CI [0.05–0.09 ng/mL] vs 0.15 ng/mL 95%CI [0.03–0.27 ng/mL], *P* = 0.004), and higher in the peripheral zone in the BPH group (0.51 ng/mL, 95%CI [0.30–0.72 ng/mL] vs 0.45 ng/mL 95%CI [0.27–0.63 ng/mL], *P* = 0.0001).

Regarding the ratio tissue/serum (Table 5), TT concentration level was lower in the tissue compared to the serum in the PCa group (ratios

TABLE 2 Comparison of compounds concentrations in serum and prostate tissue, according to the prostate malignancy

	PCa (mean 95%CI)	PBH (mean 95%CI)	$p_{randomization}$ = [10 000 iterations]
Serum (S)			
FSH (mUI/mL)	7.3 (5.10–9.15)	9.3 (5.81–11.93)	0.34
LH (mUI/mL)	4.3 (3.52–4.97)	5.7 (4.40–7.02)	0.06
SHBG (µg/mL)	3.6 (3.09–3.97)	3.3 (2.55–3.91)	0.60
BT (ng/mL)	1.2 (1.03–1.36)	1.2 (1.03–1.36)	0.88
DHEA (ng/mL)	3.0 (2.34–3.67)	2.4 (1.88–2.97)	0.20
DHT (ng/mL)	0.46 (0.409–0.510)	0.40 (0.347–0.457)	0.13
TT (ng/mL)	4.9 (4.41–5.33)	4.7 (4.11–5.33)	0.73
Δ5 (ng/mL)	0.88 (0.760–0.988)	0.74 (0.625–0.850)	0.10
E1 (pg/mL)	**33.2 (30.2–36.2)**	**43.3 (39.0–48.1)**	**0.001**
E2 (pg/mL)	25.6 (23.8–28.1)	27.5 (24.9–30.0)	0.27
DHEA-S (ng/mL)	92.9 (75.2–109.5)	84.1 (66.3–100.7)	0.46
Tissue (T)			
DHEA (ng/g)[a]	33.1 (17.2–49.0)	33.4 (16.8–50.1)	0.98
DHT (ng/g)	5.55 (5.10–6.00)	5.42 (4.88–5.96)	0.70
TT (ng/g)	**0.11 (0.05–0.18)**	**0.47 (0.28–0.66)**	**0.0002**
Δ5 (ng/g)	1.67 (1.24–2.10)	1.15 (0.80–1.43)	0.06
E1 (pg/g)	432 (251–613)	773 (178–1369)	0.25
E2 (pg/g)	**31.0 (27.6–34.4)**	**22.4 (16.3–28.4)**	**0.010**
DHEA-S (ng/g)	162 (122–202)	150 (109–191)	0.66

[a]Results obtained were given in microgram per 100 mL of sample, then converted in ng/g unit as one mL of sample weighs approximately 1 g.
Bold values are the statistically significant differences (*P* < 0.05).

TABLE 3 Comparison of compounds concentrations in central and peripheral prostate tissue, according to the prostate malignancy

	PCa (mean 95%CI)	BPH (mean 95%CI)	$p_{randomization}$ = [10 000 iterations]
Central			
DHEA (ng/g)[a]	38.0 (22.2–53.8)	35.0 (17.2–51.9)	0.78
DHT (ng/g)	5.80 (5.2–6.4)	5.60 (5.05–6.14)	0.61
TT (ng/g)	**0.15 (0.03–0.27)**	**0.45 (0.27–0.63)**	**0.004**
Δ5 (ng/g)	1.70 (1.21–2.19)	1.10 (0.82–1.48)	0.048
E1 (pg/g)	554 (290–818)	783 (184–1382)	0.49
E2 (pg/g)	**39.0 (34.3–43.7)**	**18.0 (13.6–23.1)**	**0.0001**
DHEA-S (ng/g)	175 (135–215)	145 (106–184)	0.29
Peripheral			
DHEA (ng/g)[a]	28.0 (11.3–45.4)	31.0 (15.3–46.8)	0.81
DHT (ng/g)	5.30 (4.8–5.8)	5.10 (4.41–5.80)	0.58
TT (ng/g)	**0.07 (0.05–0.09)**	**0.51 (0.30–0.73)**	**0.0001**
Δ5 (ng/g)	1.60 (1.22–2.04)	1.20 (0.86–1.55)	0.10
E1 (pg/g)	307 (188–426)	754 (126–1382)	0.18
E2 (pg/g)	23.0 (18.2–27.8)	30.0 (16.9–43.3)	0.33
DHEA-S (ng/g)	149 (106–191)	159 (113–205)	0.75

[a]Results obtained were given in microgram per 100 mL of sample, then converted in ng/g unit as one mL of sample weighs approximately 1 g.
Bold values are the statistically significant differences ($P < 0.05$).

of 0.03, 95%CI [0.004–0.043 ng/mL] vs 0.11, 95%CI [0.06–0.15 ng/mL], $P = 0.001$). Accordingly, E2 tissue concentration level was higher in the PCa group (ratios of 1.28, 95%CI [1.12–1.43 ng/mL] vs 0.80, 95%CI [0.59–0.99 ng/mL], $P = 0.0004$). Besides, adjusted tissue/serum ratio analysis did not reveal any differences neither for the precursors (DHEA, DHEA-S, and Δ5) nor for the metabolites (DHT).

Finally, adjusted comparison of potential enzymatic activity surrogate marker ratios (Table 6) underlined a significantly lower

TABLE 4 Comparison of compounds concentrations in cancerous and non-cancerous prostate tissue, according to the central or peripheral prostate zone

	Central	Peripheral	$p_{randomization}$ = [10 000 iterations]
PCa			
DHEA (ng/g)[a]	38.0 (22.2–53.8)	28.0 (10.8–45.2)	0.78
DHT (ng/g)	5.80 (5.25–6.35)	5.30 (4.8–5.8)	0.61
TT (ng/g)	**0.15 (0.03–0.27)**	**0.07 (0.05–0.09)**	**0.004**
Δ5 (ng/g)	1.70 (1.21–2.19)	1.60 (1.19–2.01)	0.048
E1 (pg/g)	554 (290–818)	307 (187–427)	0.49
E2 (pg/g)	**39.0 (34.3–43.7)**	**23.0 (18.2–27.8)**	**0.0001**
DHEA-S (ng/g)	175 (135–215)	149 (106–192)	0.29
BPH			
DHEA (ng/g)[a]	35.0 (17.6–52.3)	31.0 (15.3–46.7)	0.81
DHT (ng/g)	5.60 (5.06–6.14)	5.10 (4.40–5.80)	0.58
TT (ng/g)	**0.45 (0.27–0.63)**	**0.51 (0.30–0.72)**	**0.0001**
Δ5 (ng/g)	1.10 (0.77–1.43)	1.20 (0.86–1.54)	0.10
E1 (pg/g)	783 (184–1381)	754 (126–1382)	0.18
E2 (pg/g)	18.0 (13.3–22.7)	30.0 (16.8–43.3)	0.33
DHEA-S (ng/g)	145 (106–184)	159 (113–205)	0.75

[a]Results obtained were given in microgram per 100 mL of sample, then converted in ng/g unit as one mL of sample weighs approximately 1 g.
Bold values are the statistically significant differences ($P < 0.05$).

TABLE 5 Comparison of sexual steroids concentrations tissue/serum ratios, according to the prostate malignancy

	PCa (mean 95%CI)	BPH (mean 95%CI)	$p_{randomization}$ = [10 000 iterations]
DHEA tissue/serum	10.1 (7.0–13.1)	20.8 (3.2–33.1)	0.20
DHT tissue/serum	13.5 (11.7–15.0)	16.9 (12.1–20.5)	0.15
TT tissue/serum	**0.03 (0.004–0.043)**	**0.11 (0.06–0.15)**	**0.001**
Δ5 tissue/serum	2.1 (1.56–2.51)	1.7 (1.28–2.03)	0.23
E1 tissue/serum	13.4 (6.6–19.0)	17.6 (3.6–29.0)	0.58
E2 tissue/serum	**1.28 (1.12–1.43)**	**0.80 (0.59–0.99)**	**0.0004**
DHEAs tissue/serum	1.7 (1.54–1.89)	1.7 (1.54–1.92)	0.939

Bold values are the statistically significant differences ($P < 0.05$).

DHT tissue / E2 tissue ratio (ratios of 0.20, 95%CI [0.17–0.22 ng/mL] vs 0.64, 95%CI [0.21–1.07 ng/mL], $P = 0.002$), and also a significantly lower TT tissue/E2 tissue ratio (ratios of 0.004, 95%CI [0.002–0.006 ng/mL] vs 0.035, 95%CI [0.017–0.053 ng/mL], $P = 0.0004$) in the PCa group. Interestingly, DHT tissue/TT tissue ratio was superior in the PCa group (ratios of 139.1, 95%CI [62.6–215.6 ng/mL] vs 41.6, 95%CI [27.4–55.7 ng/mL], $P = 0.0004$).

5 | DISCUSSION

The prostate is an androgen-dependent gland. The impact of sex steroids on the initiation and development of PCa and BPH remains unclear. The relationship between the serum levels of androgens combined with their tissue concentrations has been poorly studied in PCa. Currently, reliable data on the effective concentration levels of intra-prostatic sex steroids are missing.[3,10] To our knowledge, we present herewith the first study assessing prostatic and serum concentration levels of a large panel of sex steroids, in two cohorts of patients with PCa or BPH.

Historically, most studies used immuno-assay to estimate sex steroids concentrations, whereas GC/MS is acknowledged to be the current gold standard. Previous studies using GC/MS have been based on prostate biopsies, in order to determine the androgen concentration levels, without specifying the area sampled, or using, as Pejcic et al[10] one of these areas. Finally, many of these studies did not have any control population of non-cancer patients. In our study, we avoided these pitfalls by using samples from fresh specimens (ie, radical prostatectomy for PCa, cysto-prostatectomy, or open prostatectomy for BPH), and sampled each prostate tissue in the peripheral and central areas to establish tissue concentrations of sex steroids.

We found lower TT concentrations in the prostate tissue from patients with PCa compared to those with BPH, while levels of DHT were similar between both groups. We also found higher E2 concentration levels in the prostate tissue of patients with PCa, while serum levels of DHT, TT, and E2 were similar. Therefore, we suggest that cancerous prostates consume more TT and produces more E2 than non-cancerous prostates, due to a higher aromatase enzymatic activity, while maintaining comparable DHT tissue concentrations.

TABLE 6 Comparison of enzymatic activity surrogate marker ratios, according to the prostate malignancy

	PCa (mean 95%CI)	BPH (mean 95%CI)	$p_{randomization}$ = [10 000 iterations]
Δ5 tissue/TT serum	0.42 (0.25–0.59)	0.27 (0.18–0.36)	0.11
TT serum/BT serum	4.5 (3.9– 4.8)	4.2 (3.4–5.0)	0.59
TT serum/SHBG	1.5 (1.4–1.6)	1.6 (1.4–1.8)	0.62
TT tissue/BT serum	0.10 (0.05–0.15)	0.40 (0.24–0.56)	0.0004
TT tissue/SHBG	0.05 (0.002–0.10)	0.19 (0.09–0.29)	0.007
DHT tissue/SHBG	1.8 (1.6–2.1)	2.0 (1.7–2.3)	0.44
DHT tissue/TT tissue	**139.1 (62.6–215.6)**	**41.6 (27.4–55.7)**	**0.0001**
DHT tissue/BT serum	5.3 (4.5–6.0)	5.0 (4.1–5.8)	0.57
DHT tissue/E1 tissue	0.13 (0.08–0.18)	0.11 (0.07–0.15)	0.63
DHT tissue/E2 tissue	**0.20 (0.17–0.22)**	**0.64 (0.21–1.07)**	**0.002**
TT tissue/E1 tissue	0.0027 (0.0012–0.0041)	0.0037 (0.0012–0.0063)	0.50
TT tissue/E2 tissue	**0.004 (0.002–0.006)**	**0.035 (0.017–0.053)**	**0.0001**
DHT tissue/PSA	0.87 (0.65–1.08)	2.95 (1.83–4.06)	0.0001

Bold values are the statistically significant differences ($P < 0.05$).

This hypothesis is supported by three findings arguing for a larger uptake-transformation of TT into E2 in the PCa group. First, the TT and E2 tissue/serum ratio, were, respectively lower and higher in in the PCa group (0.03 vs 0.11, $P = 0{,}001$; 1.28 vs 0.80, $P = 0.0004$). Second, the aromatase enzymatic activity surrogate markers ratio (ie, TT tissue/E2 tissue) was lower in the PCa group (0.004 (0.002 – 0.006) versus 0.035 (0.017–0.053), $P = 0.0001$), potentially due to an increased activity of the aromatase in cancerous prostates. Third, the 5 alpha-reductase enzymatic activity surrogate markers ratio (ie, DHT tissue/TT tissue) was higher in the PCa group (139.1 (62.6–215.6) versus 41.6 (27.4–55.7), $P = 0.0001$), reflecting an increased activity of the 5 alpha-reductase.

The STERPROSER trial results, which highlights the differences between patients with normal and high-volume BPH, provided some evidence for a higher activity of 5-alpha reductase enzymes, which leads to higher DHT concentration in high-volume prostates.[12] This high DHT concentration observed may reflect either higher 5-alpha reductase expression or lower expression of downstream metabolizing enzymes such as 3a-hydoxysteroid dehydrogenase. In our study, we confirm previous findings regarding the absence of difference in tissue DHT values between patients with PCa or BPH,[10,25] and provide new data regarding the increased degradation of TT to E2 within the prostate of patients with PCa. This data is of interest as Salonia et al, found a significant association between the rate of high grade PCa (ie, Gleason score ≥7 = 4 + 3) and a serum E2 level ≥50 pg/mL in a small subset of patients.[26] Interestingly, we did not find any differences regarding E2 serum level between PCa and BPH groups. We reported a lower TT concentration level and a higher E2 concentration in prostate tissue from patients with PCa compared to those with BPH. These differences persisted regardless of the prostate zone studied (ie, central or peripheral) for TT, while for E2, this difference was only observed in the central zone (39.0 [34.3–43.7] vs 18.0 [13.6–23.1], $P = 0.0001$, and 23.0 [18.2–27.8] vs 30.0 [16.9 – 43.3], $P = 0.33$, in central and peripheral zones, respectively). This observation may result from a more important aromatization in the central cancerous prostate, whereas in the peripheral prostate, the site of cancer, the metabolism of TT is oriented to ensure "normal" concentrations of DHT, to allow stimulation of the androgen receptor proliferation pathway. Despite no definite evidence supporting the concept that inflammation promotes prostate growth,[27] pathological review of all prostate samples in our study allowed to refute the hypothesis according to which difference in tissue inflammation could explain enzymatic affinity changes.

We also found lower TT concentrations in the peripheral zone than in the central zone in the PCa group. To our very best knowledge, we present the first study to investigate steroid tissue concentrations with GC/MS depending on the area of the prostate. Using immunoassays, Molher et al[28] found no significant difference in concentration of various steroids by sampling area, with the exception of androstenedione. Our results on this point constitute a starting point and need confirmation from external validation cohorts.

The strength of this study is the use of the validated GC/MS method in a systematic way, by comparing a large panel of serum and tissue sex steroids from PCa patients and a BPH control population, including the area of the prostate. Our reported values regarding TT within prostate tissue in PCa patients (0.11 [0.05–0.18] ng/g) were comparable to those assessed on radical prostatectomy specimens (0.07–0.12 ng/g).[29,30] For patients with BPH, our reported TT values (0.47 [0.28–0.66] ng/g) were comparable to those in the literature (0.43–0.81 ng/g).[10,31] Regarding DHT concentration values, our results (5.55 [5.10–6.00] ng/mL and 5.42 (4.88–5.96) ng/mL in the PCa and the BPH group, respectively) were comparable to those in the literature using GC/MS (3.33–6.18 ng/g).[10,29,30]

There are several limitations to our study. First, the lack of direct enzymatic activity analysis, which was not conceivable on our frozen samples. Secondly, other pathways of degradation besides aromatization could explain variations in tissue TT concentrations (ie, glucuronidation or sulfonation). Our research protocol was limited to the hormonal environment and did not include measurement of conjugated excretion products. Finally, we do not have SHBG tissue concentrations, which could also contribute, by differential binding, to changes in TT tissue concentration. Yet, to our knowledge, SHBG has never been assayed into prostatic tissue.

6 | CONCLUSIONS

This study has demonstrated that localized PCa is associated with lower tissue concentrations of TT and higher tissue concentrations of E2 compared with BPH, without any differences regarding DHT tissue concentrations, or TT, DHT, and E2 serum concentrations. Enzymatic activity surrogate markers could reflect a higher activity of the aromatase and the 5-alpha-reductase. These elements might reflect the ability of cancerous prostates to uptake and to transform TT into E2 in the prostatic tissue, while maintaining a "normal" DHT concentration within cancer.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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