

Master Thesis in Medicine, Nr 110

Rational Monitoring of Prostate-Specific Antigen (PSA) after Radical Prostatectomy.

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Abstract

Purpose: PSA monitoring is the main tool for urologists and practitioners to detect prostate cancer relapse after radical prostatectomy, and is recommended in current guidelines for the management of corresponding patients. PSA doubling time (PSADT) is a useful concept to assist PSA results interpretation. However, several calculation options compete, and there is no definite consensus regarding how to best follow up PSA in this condition. The aim of this retrospective observational study was to describe prognostic markers (TNM grade, Gleason score, capsular penetration), PSA trajectories and cancer relapse after radical prostatectomy in a series of patients, and to assess critically the current PSA monitoring approaches, in particular PSADT calculation.

Methods: Patients were selected from a PSA measurements database of a medical laboratory, collected over 10 years. Inclusion criteria were PSA levels monitored 3 times over 1 year or 4 times over 2 years in the aftermath of a radical prostatectomy for prostate cancer. The clinical data were collected from the medical records of practitioners (remission versus relapse status; dates of interventions; post-surgery pTNM stage, Gleason score and capsular penetration; subsequent biopsies; imaging; chemotherapy, radiotherapy or hormonal treatment). Relapse was defined by distant metastasis or biopsy or imaging showing recurrence, or by the initiation of secondary anticancer treatment. PSADT calculation was made using either the Log-slope method or the 2-points method, once including all available values of PSA, and once selecting only PSA values above 0.1 ng/mL. PSA trajectories were described by longitudinal non-linear mixed effect modelling and a variogram analysis, while their prognostic value was assessed by Kaplan-Meier survival analysis and Cox proportional hazard models.

Results: PSA trajectories are highly variable and divergent between patients, even after taking into account their T and N grade, individual clinical markers which are strong predictors of relapse. At about 2 years distance, a change in PSA becomes indicative of clinically relevant trend in a fraction of patients (5%), which increases along the time. PSADT calculated with the Log-slope method predicts relapse better than with the 2 point method. Taking into account all available PSA values is also more efficient than restricting the calculation to those above 0.1 ng/mL. Already when estimated at 6 months or 1 year postoperatively, PSADT definitely improves the prediction of relapse in addition to grade T and Nodular Invasion..

Conclusion: PSA trajectories are characterized by large variability, even accounting for known prognostic markers. Our results fully support the regular follow-up of PSA and the calculation of PSADT for prediction and detection of cancer relapse after radical prostatectomy.

Introduction

In Europe, prostate cancer is among the commonest neoplasm, with a lifelong incidence of more than 200 cases per 100000 men. In developed countries, it has become a significant public health problem.^(1,2) Radical Prostatectomy (RP) is the gold-standard treatment for localized prostate cancer,⁽³⁾ showing a benefit on survival compared to watchful waiting.⁽⁴⁾ Despite its good results, RP does not guarantee complete cancer eradication though, making secondary relapse a frequent cause of healthcare requirement, quality of life impairment and death.⁽⁴⁾

Circulating Prostate-Specific Antigen (PSA) is widely used by clinicians for prostate cancer detection, although its use for screening is the object of hot controversies, due to the important overlap between physiological levels and values indicative of tumour.^(5,6) In contrast, the use of this marker to assess curative treatment response and detect possible recurrence seems widely accepted, despite some contradictions in the literature.⁽⁷⁾ Indeed, RP is supposed to leave no glandular tissue and to almost completely suppress PSA production in the body, thus making its detection or increase highly suggestive of incomplete cancer eradication or relapse, respectively.

Prognostic markers available immediately after RP have been shown to predict the relapse of cancer. These parameters are the TNM and Gleason scores, capsular penetration and preoperative PSA level.⁽⁸⁾ Despite their significant association with the probability of relapse, they remain of limited usefulness to decide when a treatment must be reiterated.

PSA remains therefore the mostly used tool for urologists and general practitioners to detect prostate cancer relapse after RP.⁽⁹⁾ Guidelines have been established for the monitoring of post-prostatectomy PSA levels. The European Association of Urology recommends a formal serum PSA measurement calendar supplemented by digital rectal examination for routine follow-up. PSA monitoring should be performed at 3, 6, and 12 months after treatment, then every 6 months until 3 years, and thereafter on an annual basis. However, these recommendations are mostly based on experts' experience and to our knowledge, observations to support this practice have not been analyzed according to an evidence-based approach.^(10,11) Nowadays the best way to monitor PSA remains unclear and still disputed.⁽⁷⁾ A further complication lies in the definition of relapse, which itself remains unclear. Relapse can be defined by the demonstration of metastatic or local recurrence by imaging and biopsy, but most urologists acknowledge that purely biochemical cancer relapse (BCR), defined solely by PSA increase, already deserve treatment.⁽¹²⁾ Many proposals were issued to define PSA cut-offs defining BCR. For example the European Association of Urology proposes that values over 0.2 ng/mL are indicative of residual or current disease; other authors suggest cut-offs between >0.1 ng/mL and >0.4 ng/mL.^(10,13,14,15)

Further alternatives used to detect BCR are not based on absolute PSA levels, but rather on PSA kinetics over time. One such alternative is the determination of PSA doubling-time (PSADT). The use of PSADT to monitor prostate cancer relapse after RP was shown effective.(16) However, calculation methods and cut-offs are still inconsistent in the literature, leaving clinicians poorly informed and confused about PSADT utilization. (17,18)

This study is based on a retrospective analysis of a series of patients followed up after initial surgery for prostate cancer. The aim of our investigation was 1) to describe a series of patients followed up after RP, and the relationship between classical prognostic markers (TNM score, Gleason score, capsular penetration) and clinical outcome (relapse); 2) to describe the profiles of PSA measurements in these patients; 3) to assess current PSA monitoring approaches, in particular the PSA doubling time (PSADT) advised by several authors; and 4) to evaluate the predictive power of PSA monitoring regarding clinical evolution. This analysis should bring support to recommendations regarding PSA monitoring for the detection of prostate cancer relapse after radical prostatectomy.

Methods

1. Patient recruitment and data collection

This retrospective study was purely observational. Patients were selected from the database of the medical laboratory Meditest SA based in Vevey, Switzerland, who performed thousands of PSA measurements between January 2001 and December 2010 for private practitioners. Patients considered for inclusion were those having had their PSA levels monitored at least 3 times over 1 year, or 4 times over 2 years (recruitment criterion). We included those confirmed to have undergone RP for prostate cancer (selection criterion). This approach aimed to obtain data rich PSA series determined in the same laboratory. The confirmation of RP was obtained after reviewing the medical records of each patient considered for recruitment. First the patients of the laboratory database were classified by practitioner name. Then the patients from physicians who had more than ten cases in our series were selected. A first sample of three hundred patients was thus selected corresponding to eight physicians, who were contacted and accepted to open their medical records to our investigation. The study had been approved by the Ethics Committee of the Faculty of Biology and Medicine in Lausanne. The lifelong series of PSA results (uniformly expressed in ng/mL) was then recorded for the patients included. Focused reading of medical records enabled to extract relevant information database.(13) All clinical, imaging and histological exams and all treatments known to influence prostate cancer course and PSA levels (chemotherapy, radiotherapy or hormonal treatment) were recorded chronologically. The Gleason score was determined on biopsy samples and the TNM (Classification of Malignant Tumors) was based on pathology samples (pTNM) collected during RP. Gleason score and pTNM classifications were defined according to the European Association of Urology recommendations.(10,11)

The evolution of the clinical outcome of study patients was coded according to remission / relapse / progression / metastasis / persistence, each change being associated with the most accurate date based on available information. Relapse was defined by diagnosed local recurrence, distant metastasis or the initiation of secondary anticancer treatment. This definition includes cases considered as purely biochemical relapse (BCR) considered to deserve secondary treatment. We also repeated our analysis on relapse cases selectively defined based only on imaging and biopsy.

2. Population description

A description of the data was first conducted. Statistical analyses were performed using the STATA 11.1 software package (Corporation, College Station, Tex). Summary tables were used to describe the population characteristics such as age, pTNM, Gleason scores, Capsular Penetration, relapse frequency and secondary anticancer treatments. Histograms, dot-plot and box-plot graphs were generated.

3. Relationship between clinical markers and relapse

Classical tests of hypothesis like simple mean comparison tests (t-tests) and simple proportion comparison tests and nonparametric Wilcoxon rank-sum tests were applied for groups comparisons, when appropriate. Mixed-effects linear regression tests were used to analyse measures repeated in the study patients.

Regarding time to relapse, to account for censoring in our study data, it was necessary to use Kaplan-Meier survival analysis and Cox proportional hazard models. We compared competing models with likelihood ratio tests (STATA version 11) to assess the relationships between prognostic markers and relapse and estimate hazard ratios, survival probabilities and attributable risks.

4. Description of PSA profiles

Circulating total PSA was measured in Meditest laboratory during the years of follow-up by immunoenzymatic chemiluminescent sandwich assays (Elecsys (Roche), Centaur (Siemens), AU3000i (Olympus) and Dxi (Beckman Coulter)) all exhibiting functional sensitivity between 0.006 to 0.019 ng/ml (i.e. CV<20%) and intra and inter assay CV below 4 %.

It is known that a fraction of circulating PSA is enveloped by the protease inhibitor alpha-2 macroglobulin, and that this form is devoid of immunoreactivity. Another fraction is linked to the protease inhibitor alpha-1 antichymotrypsin (PSA ACT). The remaining PSA does not form complexes and is called free PSA. The latter two forms are detected by most immunoassays, and constitute what is called total PSA. (19,20,21)

PSA concentrations depend on the standard used to calibrate the test. Currently, concentrations measured by automated immunoassay systems are standardized based on the international reference standard WHO 96/670.(22)

This standardized PSA test (90% of PSA ACT and 10% free PSA) has been proposed in the mid-nineties, with the intention to mitigate the response of certain non-equimolar PSA tests. Over time, the original intent of establishing a "standard equimolar" has evolved towards the adoption of the WHO standard 96/670 as a new "standard mass" test for PSA detection. (23)

As the distribution of PSA values differed markedly from a Gaussian, we decided to transform them into logarithmic values (LogPSA) (Figure 1). Again, summary tables were used to describe the characteristics of recorded PSA levels, and histograms, dot-plot and box-plot graphs were generated.

The technique of variogram analysis was used to assess the variance of two different log-transformed measures of PSA in study patients, and its changes as a function of the time span separating both measures.(24) Over a very short time span, this variance reflects only the rapid fluctuation due to biological and analytical variability, while over a longer time span it incorporates clinically significant evolutions in the measured parameter. A variogram over several points X₁, X₂, X₃, X₄... is simply constructed as a series of variances of two points, calculated by keeping the first point as reference and taking all subsequent points into the calculation of variances:

$$\text{Variogram} = [Var(X_1, X_2), Var(X_1, X_3), Var(X_1, X_4)\dots]$$

The variance of two points is actually equal to the variance of their difference:

$$Var(DX) = \left(X_{initial}^2 + X_{current}^2 \right) - \frac{(X_{initial} + X_{current})^2}{2}$$

If there is no time trend and patients simply oscillate around their individual set point, then the expected variogram is flat and shows constant basal variability (biological + analytical). If there exists a drift in the patients, then the expected variogram increases gradually, showing how time increases the discrepancy between values successively measured in the patients. If we assume this drift to be linear, then we expect the variogram to diverge according to a parabolic curve depending only on squared time:

$$Var(DX) = Var_{basal} + Var_{slope} \cdot t^2$$

where Var_{basal} is the short-term test-retest variance (biological + analytical), amounting to twice the variance of X, and Var_{slope} quantifies the variability of individual slopes among the patient population. A

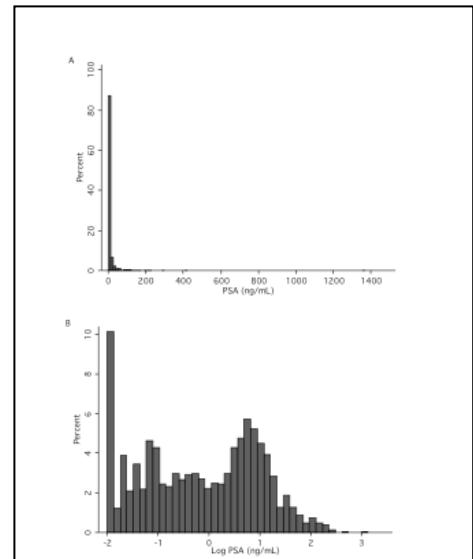


Figure 1: (A) distribution of PSA levels (ng/mL), (B) distribution of LogPSA levels (ng/mL).

family of parabolic curves can thus be fitted to the series of two-by-two variance values, using hierarchical mixed effects regression that accounts for random patient effects. Extrapolation of the average curve to time zero gives the short-term variability (biological + analytical), and we can estimate that the average time to wait for a drift leading to a variation of magnitude similar to the short-term variability.

The description of PSA trajectories was complemented by longitudinal non-linear mixed effect modelling, according to the equation:

$$PSA = PSA_{preop} (e^{-d \cdot t} + e^{g \cdot t} - 1)$$

where PSA_{preop} is the last PSA value before prostatectomy, d the rate constant for postoperative decrease, g the rate constant for late re-growth, according to model proposed by *Stein and al.* (24). This analysis was performed using the Nonmem software (version 7.1, ICON Development Solutions Division, Ellicott City, MD USA), a non-linear mixed effects modelling software tool mostly used for population pharmacokinetic-pharmacodynamic analysis, but able to fit datasets with all kinds of non-linear equations. Competing models that assumed various distributions for parameters and residual errors and incorporated clinically meaningful covariates influencing the parameters were tested by likelihood ratio tests and inspection of diagnostic plots. The model found to best describe the observations was used to generate sets of 1000 simulations for each level of predictive covariates, with random variability in parameters and errors following the assumed probability distributions. These simulations served to calculate percentiles 10, 25, 50 (median), 75 and 90 of expected spread of postoperative PSA values.

5. PSADT calculation and predictive performance

We tested the two PSADT calculation methods mostly used in the literature: the 2-point method and the log slope method.(17) The 2-point method defines PSADT as:

$$\frac{\ln(2) \cdot \Delta t}{\ln(PSA_{current}) - \ln(PSA_{initial})}$$

where Δt is the time span in years separating two PSA determinations.

The log slope method is defined by:

$$\frac{\ln(2)}{m}$$

Where the slope m is obtained by linear regression of the log-transformed PSA values over time, which can accommodate more than two points:

$$\ln[PSA_{current}] = \ln[PSA_{initial}] + m \cdot t$$

Then we applied both these formulae several times, while using different PSA cut-offs: first we used all postoperative PSA values; then we restricted to analysis to only values equal to or greater than 0.1 ng/mL, as recommended by the Sloan-Kettering Cancer Center inspired from Pound and al.(13); then to values starting at 0.2 ng/mL, inspired by the recommendations from the European Association of Urology. PSADT was also calculated at different times, i.e. six months, one, two, and three years after RP. Cox proportional hazards regression analysis was used to compare the various determinations of PSADT with regard to their additional predictive performance of cancer relapse (BCR). We used Kaplan-Meier survival analysis and Cox proportional hazard models, comparing models by likelihood ratio tests.

Test significance throughout our analyses was considered for by a p value smaller than 0.05 ($P<0.05$); 95% Confidence Intervals (CI95%) were also built up around estimates provided by model fitting.

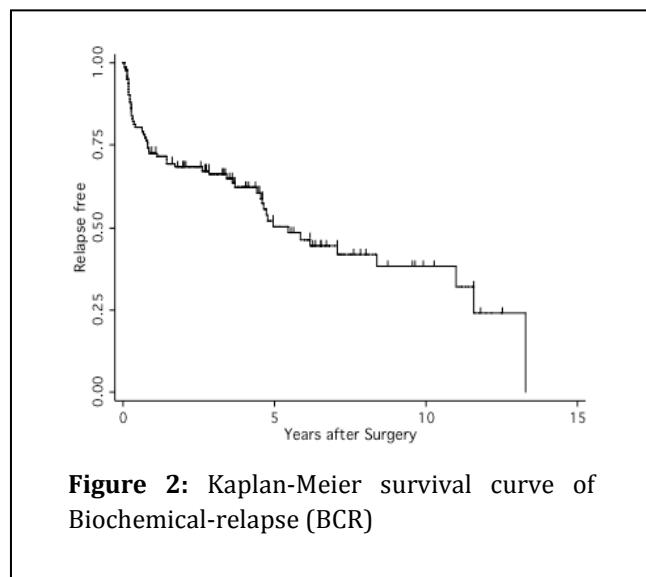
Results

1. Descriptive Analysis

1.1. Study Population

A total of 102 individuals who underwent radical prostatectomy compose our study population. Their median follow-up was 5.8 years, mean (SD) 5.0 (3.6) years, range 0.8-19 years. Their median and mean age at the time of diagnosis was 65.2 (6.75) years; the youngest was diagnosed with 50 and the oldest with 80. All clinical markers could not be established for each patient at time of diagnosis. We found 89/100 pTNM values, 93/102 Gleason scores and 79/102 biopsies information about the capsular penetration. TNM stages were quite variable between patients, with 46/102 below pT2cN0M0 on diagnosis time. On diagnosis 12/102 patients had positive nodes and one had a metastasis; the most frequent scores were pT2cN0M0 in 20 patients, followed by pT3aN0M0 in 13 and pT2aN0M0 in 12. The highest scores were pT4N0M0 and pT4N1M0 (each 1/102). Gleason scores ranged between 2 and 9; 14 patients had Gleason scores ≤ 5 , 66 between 6 and 7, and 11 ≥ 8 . A total of 212 imaging reports were reviewed during the survey in the medical records, as well as 102 biopsy reports. Fifty-two patients (52/102) had a BCR, defined by occurrence of distant metastases, local tumor recurrence on imaging or biopsies, or initiation of secondary anticancer therapy (figure 2). Among them, only thirty one (31/102) had proven relapse confirmed by imaging (MRI, CT-Scan, PET-scan) or biopsies. Twenty-one patients were thus treated on the basis of a pure biochemical relapse without establishment of further evidence of recurrence by imaging and biopsy. Twenty-three patients (23/102) developed metastases subsequently, while only one patient had metastases on diagnosis time.

Since our data are censored, this limits the interest of proportions of relapse or remission. However, our population at the end of the observation period included: 49% relapse-free patients (50/102); 21 % treated only on the basis of PSA increase (21/102); 17% treated for recurrence on imaging or biopsy exploration (16/102); 12.7% treated after PSA increase with relapse later confirmed by imaging (13/102); 1.9% never considered in remission after surgery



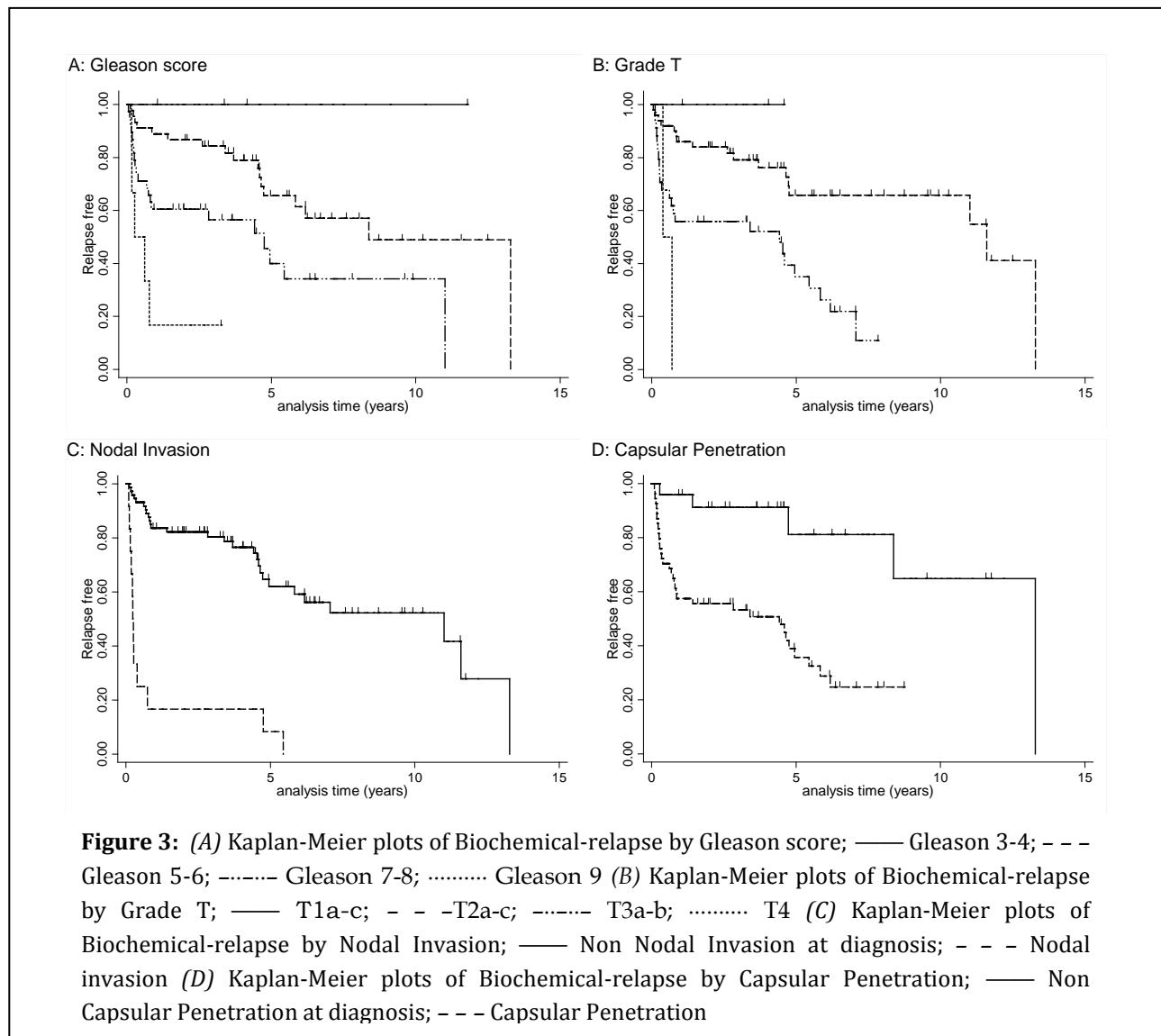
because of advanced stage cancer (2/102). It should be noted that four patients never received treatment after confirmation of recurrence, either because declining or late entry in our study leaving no time to observe the treatment. During our observation window, the mean time of BCR occurrence in recurring patients was 3.4 years (median 2.6, SD 3.1; range 0.05 to 12.2). The global description of the survival curve over the whole patient set using the Kaplan-Meier approach indicated a median [CI95%] relapse-free survival of 5.4 years [4.4 to 11.0] (Figure. 2).

Table 1: Population characteristics at the end of the study window.		
Number of patients	102	
Median follow-up years, (range)	5	(1-19)
Median age on diagnosis (range)	66	(50-80)
TNM staging classification, No. (%) of patients		
T1a	1	(0.2)
T1b	0	(0)
T1c	2	(2.2)
T2a	16	(15.7)
T2b	9	(8.8)
T2c	25	(24.4)
T3a	17	(16.8)
T3b	17	(16.8)
T4	2	(2.2)
undefined	13	(12.9)
Total	102	(100)
Gleason Score, No. (%) of patients		
2-4	4	(4.4)
5	12	(11.7)
6	33	(32.2)
7	33	(32.2)
8-10	11	(10.7)
undefined	9	(8.8)
Total	102	(100)
Capsular penetration, No. (%) of patients		
CP	54	(53)
Non-CP	24	(23.5)
undefined	24	(23.5)
Total	102	(100)
Nodal infiltration, No. (%) of patients		
N positive	12	(11.7)
N negative	73	(71.6)
undefined	17	(16.7)
Total	102	(100)
Biochemical relapse (BCR)	52	
Imagery and biopsy proven-relapse	31	

1.2. Relation between clinical markers and outcomes (univariate analysis)

Prognostic factors were first tested independently from each other to determine whether they predicted the occurrence of relapse. Firstly, age did not predict BCR ($P>0.3$, Hazard ratio (HR) [CI95%]

= 1.02 [0.9-1.1] per year). Conversely, Gleason score was highly correlated with the occurrence of biochemical or proven relapse ($P<0.001$, HR = 2.0 [1.5-2.7] per score point). Similarly, pTNM and capsular penetration strongly predicted the occurrence of BCR. To determine predictability of pTNM, we separated the TNM dimensions into: T (invasion stage), N (nodes) and M (metastases). As T is defined by the levels T1a-T1b-T1c-T2a-T2b-T2c-T3a-T3b-T4 rather than a continuous value, we sought the most suitable cut-off to create a binary variable (coded 0 or 1). T2c and T3a were clearly the cut-offs predicting BCR best ($P<0.001$; $\geq T3a$: HR = 3.81 [1.9-7.3]; $\geq T2c$: HR = 4.99 [2.0- 12.1]). For the sake of simplicity we used the cut-off $\geq T3a$ for T. with a predictive value was found as well for positive nodal invasion on diagnosis (N) ($P<0.001$; HR = 8.24 [3.9- 16.9]). Only one patient was diagnosed with metastases, not enabling to evaluate a predictive power. Capsular Penetration, determined for seventy-eight patients, also strongly predicted the occurrence of BCR ($P=0.001$; HR = 5.72 [1.9-16.5]). The differences in survival curves associated with parameters recorded at the time of initial diagnosis are striking (Figure 3).



1.3. Prediction of outcome by clinical markers (multivariate approach)

The many clinical makers separately found to predict the outcome are however intercorrelated with each other, calling for their combination in a multivariate analysis. Combining T score ($T \geq T3a$) with nodular invasion (N), both markers maintained a high level of prediction ($P=0.0001$ for both; $\geq T3a$: HR = 5.1 [2.4-10.9]; N: HR = 3.9 [1.9-8.2]). Adding Gleason score to T and N made Gleason score lose significance ($P=0.12$; HR = 1.36 [0.9-2.0]). Trying to delineate cut-offs on Gleason score did not improve its predictive value. The same happened on combining capsular penetration with T and N, which also lost its significance ($P=0.07$; HR = 3.8 [0.8-18.2]). We concluded that in our study population, the only markers association remaining significant was the combination of T ($\geq T3a$) and nodular invasion (N) on diagnosis (Figure 4).

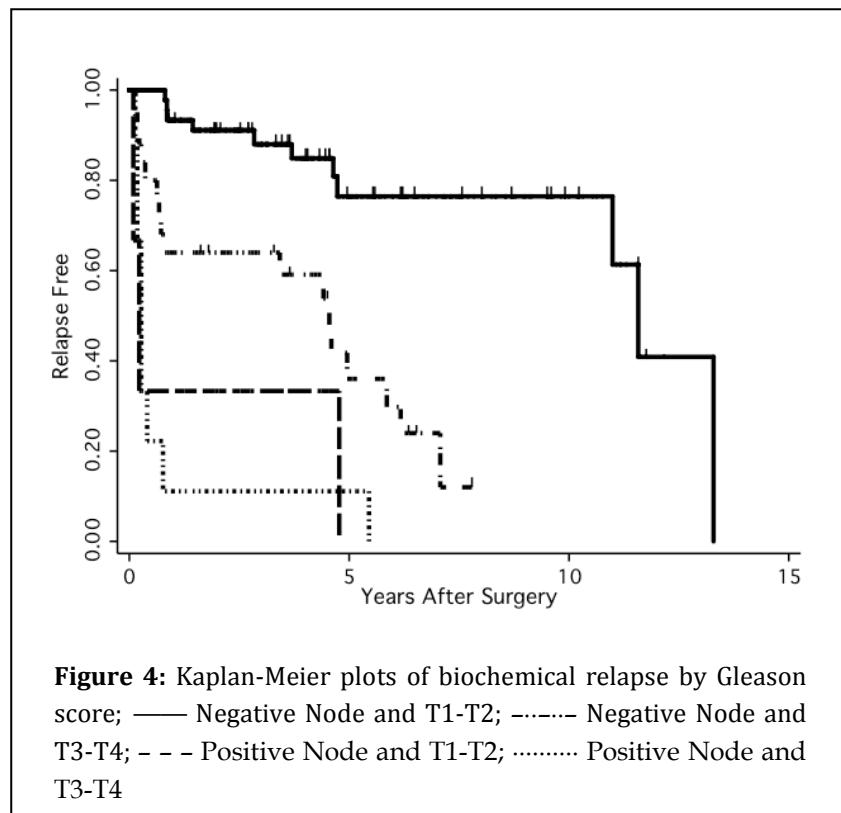


Figure 4: Kaplan-Meier plots of biochemical relapse by Gleason score; — Negative Node and T1-T2; - - - Negative Node and T3-T4; - - - Positive Node and T1-T2; Positive Node and T3-T4

2. Assessment of PSA post-operative Monitoring

2.1. Time of postoperative PSA determinations

The number of PSA measurements by patient varied between 5 and 45, the median for the population was 16 determinations per patient. The PSA levels spanned from 0.01 to 1364 ng/mL; despite the existence of high values the median of PSA levels was 0.3 ng/mL and the mean 0.34 ng/mL (Table 2).

Table 2: PSA and PSADT levels measurements characteristics		
Total of PSA measurements	653	
Median PSA measurements pro patients, (range)	16	(5-45)
Mean of total PSA levels (ng/mL), (range)	0.3	(0.01-1363)
Median PSA decrease (%) after Radical Prostatectomy, (p25; p75)	-98%	(-95%; -99%)
Median time (month) past after Radical Prostatectomy (range) for:		
1 st PSA measurement	1.3	
2 nd PSA measurement	5.3	
3 rd PSA measurement	10.3	
Total PSADT log slope method measurements	438	
Total PSADT 2-points method measurements	436	
Median PSADT log slope method in month (p25; p75)	8	(-5.7; 23.8)
Median PSADT 2-points method in month (p25; p75)	3.2	(-1.9; 14.6)

After radical prostatectomy we observed a PSA decrease, by a mean (SD) of -91% (24%). This decrease was evaluated for 96 patients with early postoperative PSA determination. The median of the decrease was about -98%.

The levels of PSA measured out of relapse were compared with those measured on relapse detection. A multilevel regression analysis was used to account for both inter-patient and intra-patient variability, and revealed a difference of 0.875 in logarithms, corresponding to a 7.5-fold increase ($p<0.0001$); this difference showed an inter-patient variability of 3.4 fold, while the inter-patient variability in the baseline was 6.2 fold, and the residual (intra-patient) variability 4.2 fold (Figure 5).

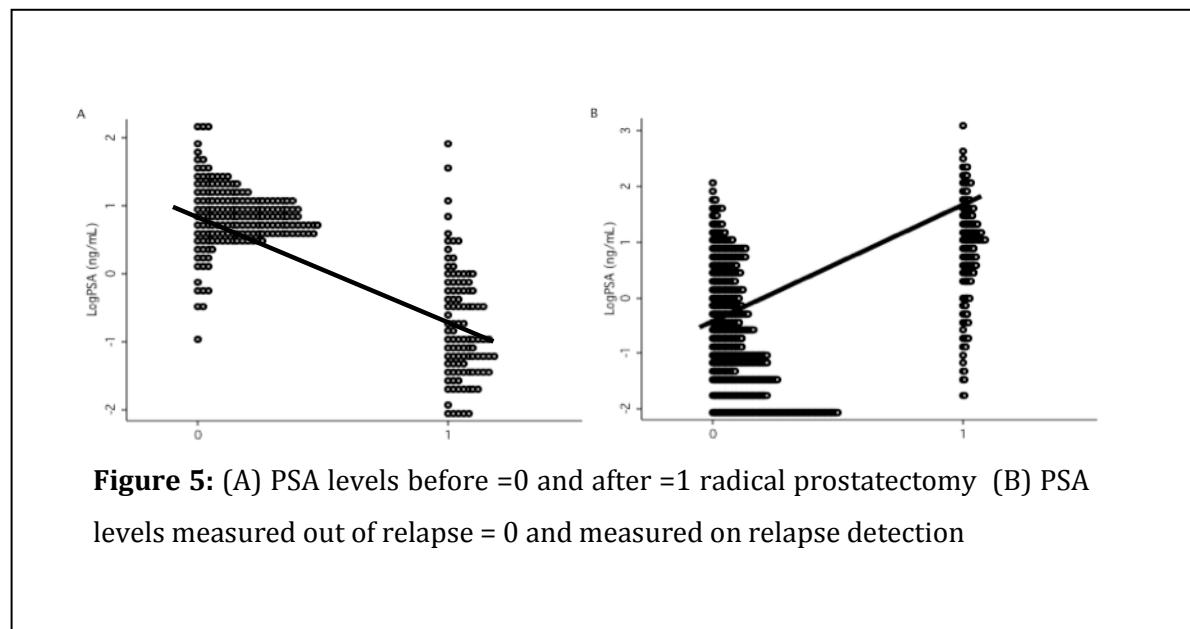


Figure 5: (A) PSA levels before =0 and after =1 radical prostatectomy (B) PSA levels measured out of relapse = 0 and measured on relapse detection

The European Association of Urology recommends postoperative PSA level determination at 3, 6 and 12 months. Our data actually indicated an median of the first control time at 1.3 month (40 days) after RP (IQR; 1 to 2.8 months). In other words, seventy-five percent of PSA measurements were requested before three months. Regarding the second and third PSA measurements, the practitioners behaved closer to the European recommendations, the second PSA was requested at a median time of 5.3 months after RP (IQR; 3.2 to 8 months) and for the third one at 10.3 months after RP(IQR; 10.3 to 14 months).

2.2. Postoperative PSA trajectories

The individual postoperative PSA trajectories observed before institution of any secondary treatment were modeled according to the equation proposed by *Stein and al* (24). PSA trajectories were highly variable and divergent between patients. The best model that we found to describe the population dynamics of PSA accommodated log-normally distributed (i.e. proportional) variability on 3 parameters, namely the preoperative PSA level, the relative postoperative decrease rate constant and the late regrowth rate constant. A better fit was obtained on introducing covariance terms between the parameters in the population, thus assumed to follow a fully specified log-trinormal distribution. The residuals (differences between modeled and observed values) were considered to follow a mixed additive and multiplicative error distribution. A systematic search for influential covariates on the regrowth rate constant identified the T score (dichotomized into $T < 3$ versus $\geq T3a$) and the N score (0 versus 1) as statistically significant predictors of PSA trajectories, in line with the results of our relapse-free survival analysis. The model identified a very steep postoperative decrease in PSA with a rate constant (d) of 50.9 year^{-1} , corresponding to a half-life of 5 days. Patients with scores of $T < 3$ and

$N=0$ had an average regrowth rate of 0.01064, corresponding to an average doubling time of about 65 years; however, a salient variability of 210% affected this parameter. A T score of T3a or more would divide this doubling time by 5.14, and a N score of 1 would further divide it by 11.3 (Table 3).

Table 3: Population parameters characterizing postoperative PSA trajectories
(RSE= relative standard errors)

Parameter	Population average (RSE)	Population variability (RSE)
$\text{PSA}_{\text{preop}}$ (ng/mL)	6.99 (11%)	84% (19%)
d (year $^{-1}$)	50.9 (10%)	51% (25%)
g (year $^{-1}$)	0.01064 (9%)	210% (21%)
$(T \geq T3a)$ on g	$\times 5.14$ (52%)	
$(N=1)$ on g	$\times 11.3$ (142%)	

A global graph of the fitted individual trajectories superimposed to the observations (figure 6) shows the very important variability in the evolution of postoperative PSA. Graphs were also generated based on simulations, showing the typical trajectories for the 4 combination of T and N scores, surrounded by 25%-75% and 90% prediction bands confirming the persistence of wide variability even after accounting for both scores (figure 7).

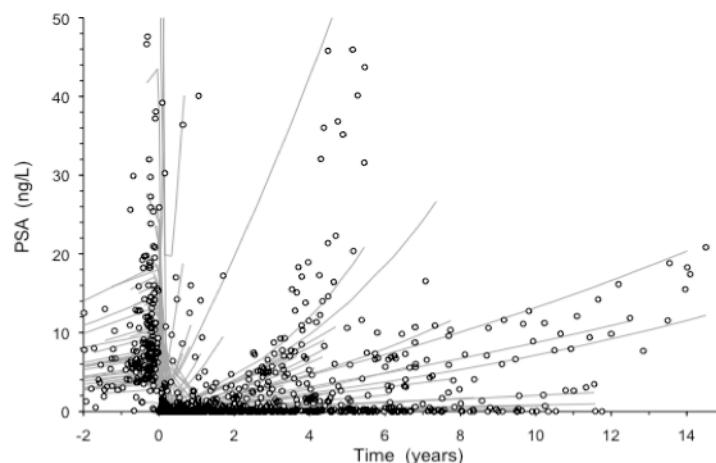
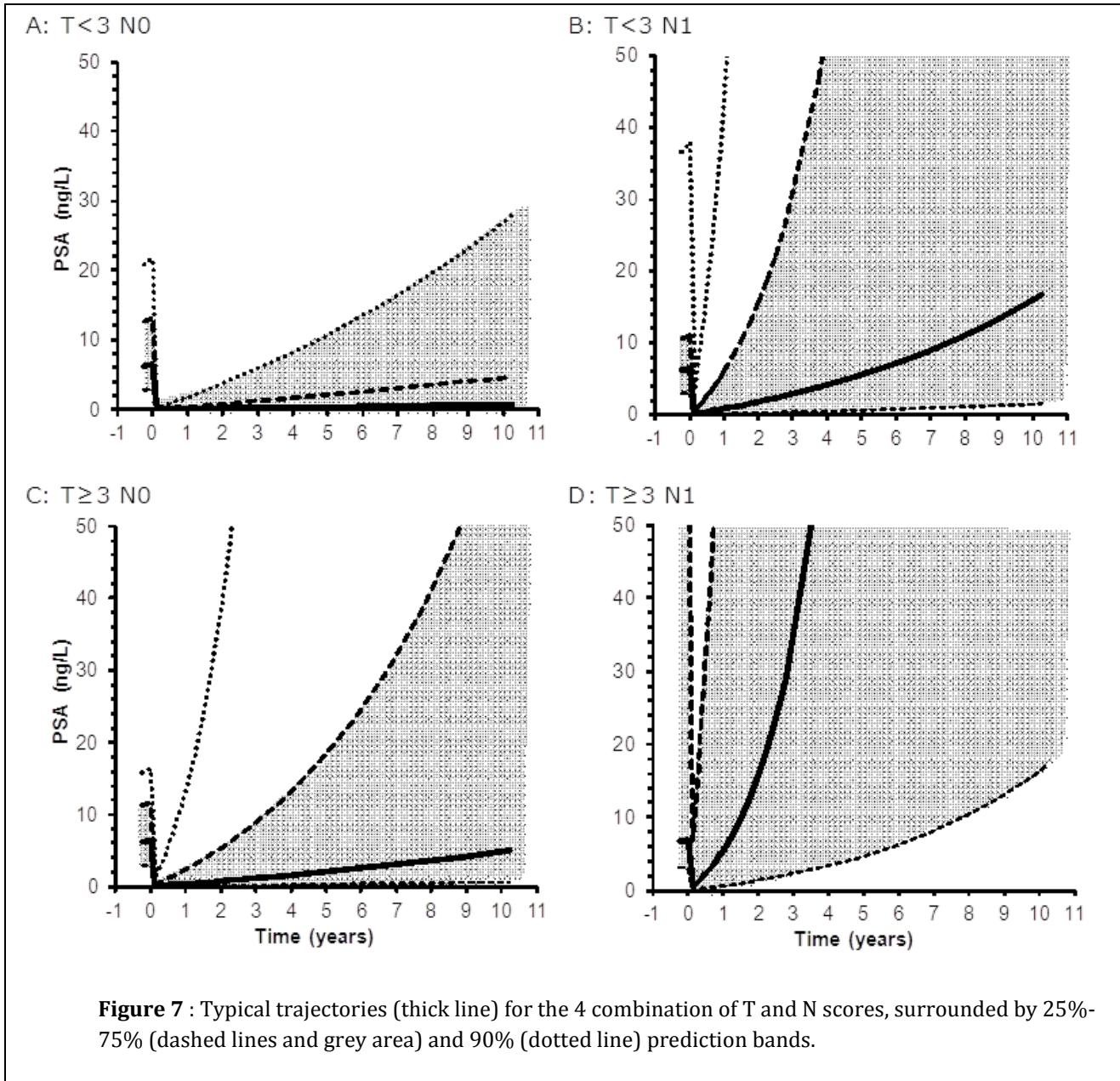


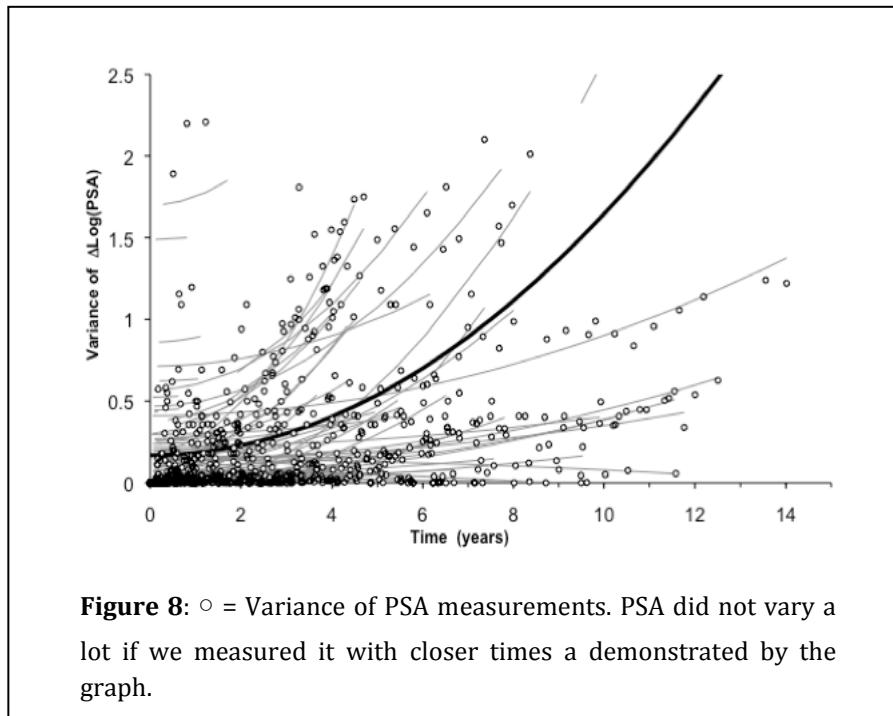
Figure 6: PSA trajectories are highly variable and divergent between patients, even taking into account T and N status.



2.3. Variogram of PSA evolution

Variograms to assess the variance PSA levels were constructed as well, to demonstrate the increase in two-point variance in Log(PSA) levels over time, the first measurement corresponding to the earliest postoperative PSA value. (Figure 8) Again, the analysis selected only PSA values preceding any secondary treatment. Under the assumption of linear drifts in LogPSA values, the variogram was assumed to diverge mathematically according to a parabolic curve depending only on squared time. A multilevel regression analysis found a short-term test-retest variance ($\text{Var}_{\text{basal}}$) of 0.17, corresponding to 121% short-term variability in the original PSA measure, and a quadratic slope ($\text{Var}_{\text{slope}}$) 0.0148, indicating that only after 3.4 years would an increase in PSA have a probability of 50% or more to

correspond to a definite increase rather than to short term test-retest variability. But here again, this global estimate is associated with very important differences among individual patients, which limits its general applicability. Actually the short-term variance spreads with an SD of 0.31 among patients, and the quadratic time slope with an SD of 0.0196, suggesting that some patients have a steady variance on the long term, and some others a rather quickly increasing variance, which is in line with the important variability found in individual PSA trajectories. For example, 5% of the patients would need less than 1.9 years for their increase in PSA to exceed the short-term test-retest variability.



2.4. PSA responsiveness to re-intervention

The purpose of our work was not to describe and to examine the response of PSA to treatments such as radiotherapy and hormonal therapy. However, we mention the impact that re-intervention can have on PSA levels. The median PSA decrease observed in 36 patients receiving radiotherapy was -40% (IQR; +1.32% to -73%), and the median of PSA decrease in 92 patients receiving hormonal therapies was -83% (IQR; -25% to -97%). This impact of PSA was assessed across both proven-relapse and BCR cases.

3. Analysis of PSA doubling time

3.1. Determination of PSA doubling time (PSADT)

Both methods of PSADT calculation, namely the 2-points and the log slope method were tested. We applied several options for determining the doubling time in order to compare them. The PSADT values appeared to follow a markedly non-normal distribution, making it difficult to reveal any relationships between the occurrence of relapse and doubling time by using the values in the original scale. To improve this, we applied the inverse transformation to PSADT ($1/\text{PSADT}$), thus obtaining a more Gaussian distribution as shown by the two graphs below (Figure 9). The PSADT values, provided in years, make higher values reflect slow progression, lower values rapid progression, but negative values a decrease in tumor secretion. Conversely, higher values of inverse PSADT (in year $^{-1}$) indicate rapid progression, lower values slow progression, zero an absence of progression, and negative values a regression, which is advantageous for grouping and calculations, besides their better behaved distribution. However, we transformed back our results into the PSADT scale (in months) to express them according to usual practice.

PSADT was first determined using the 2-points method. We obtained 438 values on including all PSA values, with a median doubling time of 3.2 months (IQR; -1.9 to 14.6). Selecting higher PSA concentration cut-offs, we obtained 355 values for a cut-off $\geq 0.1 \text{ ng/mL}$ with a median of 3.4 months (IQR; -1.1 to 12.5 months), and 324 values for a cut-off $\geq 0.2 \text{ ng/mL}$ with a median of 3.2 month (IQR; -1 to 12.3 months).

Secondly, PSADT was determined using the Log-slope method. Including all PSA values gave a median doubling time of 8 months (IQR; -5.6 to 23.8 months). Selecting higher PSA level cut-offs, we obtained 245 values for a cut-off $\geq 0.1 \text{ ng/mL}$ with a median of 12.2 months (IQR; 6 to 31 months) and 210 values for a cut-off of $\geq 0.2 \text{ ng/mL}$ with a median of 12 months (IQR; 6.6 to 26.4 months).

Increasing the PSA cut-off leaves less available PSA measurements to determine PSADT and tends to select out shorter estimates of PSADT.

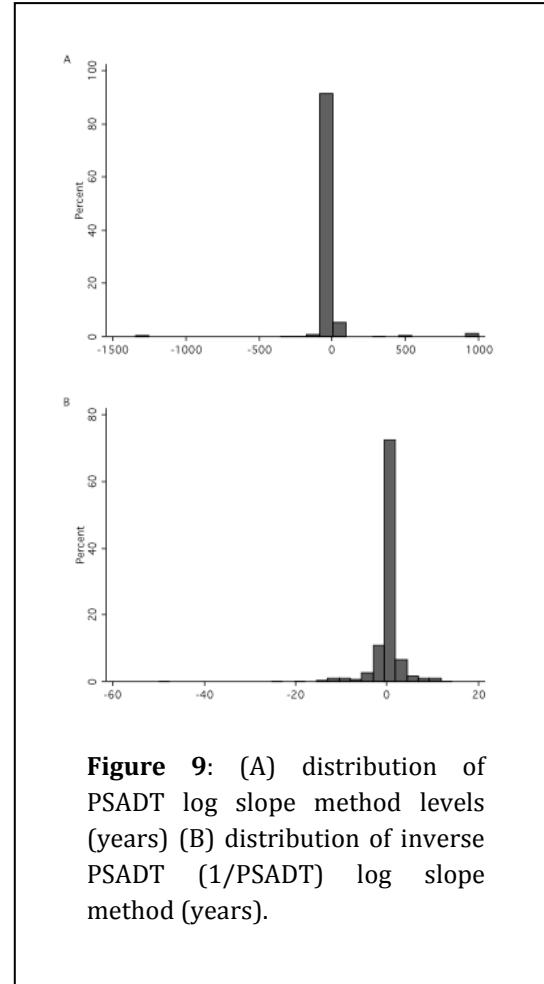


Figure 9: (A) distribution of PSADT log slope method levels (years) (B) distribution of inverse PSADT ($1/\text{PSADT}$) log slope method (years).

3.2. PSADT log slope method versus 2-points method (univariate approach)

When we compared both methods of PSADT calculation, regardless of the follow-up time and without using any cut-off of PSADT, we observed that PSADT calculated with the Log-slope method predicted BCR ($P<0.0001$, HR [CI95%] = 1.83 [1.4-2.4]) than PSADT calculated with the 2-point method ($P=0.009$, HR = 1.4 [1.1-1.8]). The explanation is that the Log-slope method calculates PSADT taking into account all PSA levels before the last PSA measurement, compared to the 2-point method that calculates PSADT considering only the first and last PSA levels. Because of this characteristic, the Log slope method appears more suitable to predict BCR after radical prostatectomy.

Applying a cut-off to include PSA levels into PSADT calculation did not improve BCR prediction. Dropping out PSA values below 0.1 ng/mL or 0.2 ng/mL decreased the significance of predictions based on PSADT compared to including all PSA values. Using the 2-point method, the selection of PSA values above 0.1 ng/mL decreased the predictive power of PSADT ($P=0.071$, HR = 1.3 [1-1.7]), and the selection of values over 0.2 ng/mL even further ($P=0.095$, HR = 1.26 [1-1.7]), in comparison to the inclusion of all PSA values ($P=0.009$). The same was observed using the Log-slope method, if we restricted the calculation to PSA values over 0.1 ng/mL ($P=0.003$, HR = 2.5 [1.4-4.6]) and over 0.2 ng/mL ($P=0.005$, HR = 2.3 [1.3-4.1]), in comparison to keeping all PSA values ($P<0.0001$). Thus, applying PSA cut-offs to PSADT calculation just worsened its predictive value, some predictions being still significant though.

4. Predictive value of PSADT regarding cancer relapse

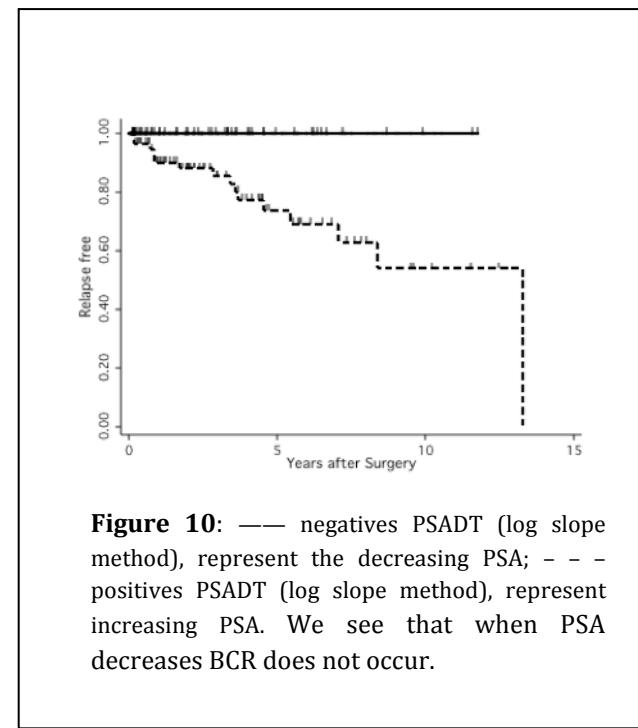
4.1. PSADT predictive value at 6 month, 1 year, 2 years and 3 years after RP (univariate approach)

As mentioned above, the best method for calculating doubling time was the Log-slope method. Determining PSADT on results obtained within 6 months after operation significantly predicted BCR occurrence on the long term ($P=0.005$, HR [CI95%] = 1.2 [1-1.3]). Similarly, BCR was significantly predicted by PSADT determined with 1 year after operation ($P=0.001$, HR = 1.4 [1.1-1.7]), 2 years ($P=0.033$, HR = 1.7 [1-2.8]) and 3 years ($P=0.001$, HR = 2.6 [1.4-4.6]). Selecting only the PSA values over 0.1 ng/mL again decreased its predictive power (for example PSADT determined with the Log-slope method at 1 year using only values ≥ 0.1 ng/mL gave $P=0.475$ and HR = 0.95 [0.9-1.1]).

4.2. Predictive value of PSADT cut-offs (univariate approach)

Although PSADT determined by the Log-slope method was highly significant to predict BCR after radical prostatectomy, it is a continuous valued marker possibly difficult to use in practice. Therefore, we tried to determine whether specific doubling time cut-off would be as much predictive of BCR, and more convenient regarding clinical use. We first calculated the predictive value of negative PSADT estimates (which indicate a decrease in PSA) versus positive ones (which indicate a rise in PSA). Unsurprisingly, positive PSADT was strongly predictive of a relapse compared to negative PSADT (extremely high HR estimate). Notice that this approach turned out to be completely specific regarding relapse in our patient population (Figure 10). Then we decided to test further doubling time cut-offs, namely 3 months, 6 months, 12 months and 24.

All cut-offs were significant for forecasting BCR at 6 months and 1 year, the best cut-off was 3 months of doubling time (6 months: $P=0.005$, HR [CI95%] = 5.6 [1.7-19.1]; 1 year: $P=0.010$, HR = 5.4 [1.5-19.2]). At 2 years and 3 years the best cut-off was 12 months of doubling time: (2 years: $P=0.010$, HR = 3.8 [1.4-10.7]; 3 years: $P<0.001$, HR = 5.4 [3.2-30.6]).



4.3 Added value of PSADT in outcome prediction (multivariate approach)

As demonstrated above, PSADT calculated with the Log-slope method predicted the outcome significantly as single predictor most of the times. But in order to establish its real utility, it was necessary to check whether PSADT added something to clinical markers such as T ($\geq T3a$) and nodular invasion N(=1), which were the clinical markers found to best predict relapse. We therefore combined PSADT with the clinical markers T $\geq T3a$ and N=1, using an assumption-nested likelihood ratio test. Patients with unknown clinical markers T or N were excluded from the calculation (19/102).

All inverted PSADT log slope values added to T $\geq T3a$ and N=1, taken with no time cut-offs, improved the prediction based only on clinical markers ($P<0.0001$, HR [CI95%] = 1.7 [1.3-2.2] per year $^{-1}$). A significant prediction benefit was observed as well for PSADT calculated with the 2-point method, but with a smaller P value ($P=0.03$). Taking all PSADT values (log slope method) calculated without using

PSA below 0.1 ng/mL had still a significant, however less marked predictive effect ($P=0.002$). We also analyzed in a multivariate approach the predictive value of PSADT (Log-slope) at 6 month, 1 year, 2 years and 3 years after RP, including all PSA values in PSADT calculation.

PSA doubling time evaluated over the 6 first months postoperatively still contributed to BCR prediction after taking into account the clinical markers T and N ($P=0.016$, HR = 1.1 [1-1.3] per year $^{-1}$). The best PSADT cut-off determined 6 months post surgery was 3 months doubling time ($P=0.038$ HR = 5.4 [1.3-22.3]).

PSADT measured over the first year after surgery contributed similarly to BCR prediction, with a Hazard ratio of 1.3 per year $^{-1}$ ($P=0.033$ HR = 1.3 [1-1.7]). We were unable to determine a cut-off regarding the doubling times at 1 year if we take into consideration the interquartile range of PSADT log slope method (IQR; 5.7 to 23.8 months). Nonetheless the cut off of 60 months was determined to the first year post surgery ($P=0.038$).

PSADT at 2 and 3 years post surgery did not improve BCR prediction compared to only T score and nodal invasion ($P=0.25$ at 2 years, $P=0.83$ at 3 years). However, a cut-off of 8 months doubling time at 2 years and 24 months doubling time at 3 years improved the BCR prediction ($P=0.047$ for 8 months at 2 years and $P=0.0005$ for 24 months at 3 years) ([8 months: HR = 3.7 [1-12.8]; 24 months: 8.1 [2.1-30.7]]).

Discussion

This retrospective study recorded longitudinal series of PSA determinations in 102 unselected patients followed up after radical prostatectomy for prostate cancer. It found again the relationships already known between classical prognostic markers (TNM score, Gleason score, capsular penetration) and the probability of relapse or duration of relapse-free survival. The profiles of PSA measurements could be described according to a non-linear mixed effect model, which confirmed the predictive role of TNM score. A wide variability remained however salient among individual PSA trajectories, bringing definite justification to regular monitoring of this biomarker after surgery. The strategy currently recommended for PSA monitoring makes sense. There is a definite advantage to examine the kinetics of PSA rather than its mere levels, which can be made through the calculation of PSA doubling time (PSADT), as advised by several authors. Our data indicate that the Log-slope calculation method is preferable to the 2-points method, and that the inclusion of all PSA values is preferable to the selection of values above 0.1 or 0.2 ng/mL suggested by some authors. Even in the early postoperative period (6 months or 1 year), PSADT definitely improves the prediction of prostate cancer relapse after radical prostatectomy.

The characteristics of our population do not markedly differ from large populations cohorts previously described. A retrospective review of a surgical series including 1997 men between 1982 and 1997 operated by a single surgeon for clinically localized prostate cancer with a median follow-up of 5.3 years, found only 18% of relapses (13), which is less than our 51%. This is however explained by this series containing only 7.6% of patients with T grade T2c or higher, in comparison to 40.5% in our series. Only 17% of our patients with a T grade below T2c experienced cancer relapse.

It had been repeatedly shown that prognostic markers such as TNM grade, Gleason score and Capsular penetration predict significantly the occurrence of relapse. Due to intercorrelation between those markers, in our limited case series T grade and nodular invasion were the factors best predicting the risk of relapse, whereas Gleason score and capsular penetration did not bring further refinement to this prediction. The cut-off T3a appears to be adequate to split patient into groups in terms of relapse prediction, while the presence or absence of node invasion delineates further subgroups. However, individual PSA trajectories inside these subgroups remain highly variable and divergent, thus leaving room for the contribution of PSA monitoring to better predict relapse.

The superiority of the Log-slope method for post surgery PSA monitoring is mainly due to the inclusion of all available measurements, while the 2-point method takes into account only the first and latest PSA in order to calculate a doubling time, thus leaving more influence to "noise", i.e. errors related to both analytical methods and short-term biological fluctuations.

The Sloan-Kettering Cancer Centre, based on the work of Pound *et al.*,⁽⁹⁾ offers online prostate cancer calculators for PSA doubling time. Such tools can be used to calculate PSA doubling time, in months or years in order to predict relapse. Physicians only have to introduce dates and PSA values to determine PSADT, calculated using the log slope method. Only the ~~PSA~~ ng/mL can be inserted in the calculation tool, though. In contrast, our work indicates that it is advantageous to consider all PSA values even those below 0.1 ng/mL. Indeed, our PSADT estimates including all values of PSA predicted relapse significantly better. This would suggest that PSA measurements are reliable even for very small values. In our case however, we have exclusively selected PSA values coming from the same laboratory, and our observations might not be applicable to series of PSA measures produced by different laboratories.

PSADT improved prediction also in association with clinical markers such as T grade and nodular invasion. The establishment of doubling time cut-offs, as suggested by others (25), did not improve the predictive effectiveness of PSADT.

This work has several limitations. First, it included a limited number of patients, with a possible impact on its ability to detect clinically significant trends with sufficient statistical significance. We believe that our study population has little chance to suffer from a selection bias, however this is impossible to prove, as sociologically different patients (e.g. with insufficient follow up or complicated evolution) may not have been recruited with our criteria. The duration of the investigation window may not have been long enough to observe late relapses, due to censored data. This censorship may also be due to patients who have died of causes unrelated to CP or patients who left the laboratory during the observation time. The accessibility of medical records by the attending physician may also have induced subtle selection biases and undermined the representativeness of the sample. All patients came from the same geographical area and were operated by a small group of surgeons. Although such a study minimizes the impact of surgeons regarding outcomes (it was said that the only surgical impact is the inadvertent capsular incision during the operation), the modus operandi of western Swiss surgeons may certainly differ from other parts of the World, and thus influence the occurrence of relapses.⁽²⁶⁾ The value of PSA can be further influenced by medication, patient lifestyle (bicycle, sport) or by non-resected normal tissue.^(27,28,29)

As the patients followed up over the most prolonged durations ended up with relapse (figure 1), this suggests that the relevant question for PSA monitoring is not whether a relapse will occur or not, but rather when it will occur. In that aspect, another limitation of our study was that the predictive value of PSA monitoring was evaluated against an outcome that included itself the evolution of PSA in its definition. Moreover, in 40% of our relapse cases, the diagnosis of relapse relied solely on PSA values, making the evaluation of PSA monitoring a quasi circular problem in such situations. However, the evaluation of a monitoring strategy is not limited to its ability to predict an outcome. The variogram approach is another way to determine whether the frequency of monitoring is in accordance with its ability to detect clinically relevant trends. In this regard, the frequency of PSA monitoring advised by current recommendations seems not markedly exaggerated, due to the clinically relevant information content of this biomarker in a situation dominated by marked inter-patient variability in trajectories.

In conclusion, our results essentially support the regular follow-up of PSA and calculation of PSADT for detection of cancer relapse after radical prostatectomy.

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