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Preoperative Insulin-Like Growth Factor-Binding Protein-3 (IGFBP-3) Blood Level Predicts Gleason Sum Upgrading

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BACKGROUND. About 43% of men with low Gleason grade prostate cancer (PCa) at biopsy will be finally diagnosed with high-grade PCa at radical prostatectomy (RP). Gleason sum at RP is a good indicator of biochemical recurrence and poor clinical outcome. Therefore, there is a need to improve clinical evaluation of PCa aggressiveness in order to choice appropriate treatment. To this aim an easy-available tool is represented by circulating biomarkers. Among these, the best candidates are some molecules involved in PCa pathogenesis such as IGFBP-2 and IGFBP-3, IL-6, and its soluble receptor (SIL-6R).
METHODS. In this study, we evaluated the ability of preoperative IGFBP-2, IGFBP-3, IL-6, and SIL-6R serum levels to predict Gleason score upgrade in 52 PCa patients.
RESULTS. We found that IGFBP-3 median levels were significantly lower in patients who showed Gleason upgrading from biopsy to RP ($P = 0.024$). We also found an association between biopsy T-stage and Gleason Upgrade ($P = 0.011$). Using multivariate logistic regression model, we demonstrated that the association of IGFBP-3 serum levels together with biopsy T-stage and biopsy Gleason score was useful to calculate a prognostic risk score. ROC curve analysis of risk score showed a good ability to predict GSU (AUC = 0.81; 95% CI 0.69–0.93).
CONCLUSIONS. Our results suggest that preoperative IGFBP-3 circulating levels determination may be useful to predict Gleason score upgrading alone and/or in combination with biopsy T-stage and biopsy Gleason score. *Prostate 72: 100–107, 2012.*

KEY WORDS: **IGFBP-2; IGFBP-3; IL-6; sIL-6R; PSA; Gleason upgrading**

INTRODUCTION

Prostate cancer (PCa) is the prevalent malignant disease in men in most Western countries [1]. Biopsy Gleason score together with stage, serum prostate-specific antigen (PSA) and tumor volume is used to carefully select men with low-risk PCa that will likely benefit from treatment other than radical prostatectomy (RP) such as active surveillance (AS) or low dose brachytherapy (LD-BT) [2,3]. Many efforts have

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been made in order to maximize cancer detection at initial diagnosis and to increase the concordance of Gleason scores of needle biopsies and prostatectomy specimens especially improving biopsy techniques [4–6]. Unfortunately, many men (about 43%) with low-grade PCa at biopsy will be finally diagnosed with high-grade PCa at RP. Therefore, RP Gleason score is better than biopsy Gleason score as indicator of biochemical recurrence and poor clinical outcome [7–10], advising that Gleason sum upgrading (GSU) could be clinically relevant to estimate the probability of a more aggressive variant of PCa [11]. At present, to predict Gleason sum the model includes clinical stage, biopsy Gleason, and serum PSA [12]. However, the relationship between PSA and tumor grade is not clear because tissue PSA concentration decrease with increasing Gleason sum [13]. Free PSA (F-PSA) testing as an adjunct to total PSA has improved the diagnostic performance in men with total PSA levels of 4–10 ng/ml, but data on the usefulness of percentage of F-PSA as predictor of aggressive disease progression are inconclusive [14,15]. Several alternative biomarkers have been proposed to supplement or replace PSA in order to predict the natural behavior of the tumor [16–19]. The candidate biomarkers are molecules involved in biological pathways thought to be important in pathogenesis and progression of PCa and potentially able to refine clinical decision making. These markers include insulin-like growth factor-binding protein-2 (IGFBP-2) and -3 (IGFBP-3), interleukin-6 (IL-6), and its soluble receptor (sIL-6R). Insulin-like growth factors (IGFs) have mitogenic effects on epithelial cells [20] and are protected from degradation by their own binding protein; moreover, IGFBPs transport IGFs to distant effector sites and modulate the interaction with the surface receptors, affecting the cellular response [21]. Several studies showed the association of local expression of IGFs, IGFBP-2, and IGFBP-3 with tumor grade and pathologic stage in breast, lung, colon, and PCa [22–25]. IL-6, a cytokine involved in immunological and inflammatory response, has been shown to have a role in proliferative and invasive potential of cancer cell [26,27], increasing angiogenesis, invasion [28,29], and promoting immune escape by tumor cells [30]. The soluble form of IL-6 trans-membrane receptor (sIL-6R) is able to bind the cytokine and potentiate the signal transduced or activate it in cells that do not express the receptor [31]. This kind of signal transduction, called IL-6 trans-signaling (IL-6TS) has a major relevance in the pathogenesis of chronic inflammatory and malignant disease [32–34]. In this study, we used a database prospectively collected during a short period to evaluate if the preoperative serum levels of IL-6, sIL-6R, IGFBP-2, and IGFBP-3 may be used to predict GSU. These parameters may be useful when consulting patients before treatment decision are made.

MATERIALS AND METHODS

Patients

During a 1-year period, a total of 52 subjects with PCa, age ranging from 51 to 75 (median = 64 years), were enrolled at our institution. Inclusion criteria were no evidence of active infection or inflammatory disease, no neo-adjuvant androgen therapy, PSA $<$ 20 ng/ml, clinical stage $<$IIc. Patients were classified by clinical stage, pathologic Gleason sum, and significant GSU. Significant GSU is defined as a Gleason sum increase between biopsy and RP either from $\leq$6 to $\geq$7 or from 7 to 8 [35]. Clinical stage was assigned by the attending urologist according to the 2002 TNM system. Between 14 and 16 needle biopsy cores were obtained under transrectal-ultrasound (TRUS) guidance: 40 (74%) had 14 cores taken and 15 (27%) had 16 cores taken. Primary and secondary Gleason score were assigned by the same pathology. All prostatectomy specimens were processed according to the Stanford protocol and were also graded according to the Gleason system.

Measurement of Biomarkers in Serum

Preoperative serum samples were collected before digital rectal examination (DRE) and TRUS. Blood was collected into non-heparinized tubes and serum was separated within 1 h of blood collection. The serum was stored at -80°C and then thawed just prior to testing. Serum levels of PSA, F-PSA, IL-6, and IGFBP-3 were measured using the Immulite 2000 automated assay (DPC, Los Angeles, CA). Concentration of sIL-6R (R&D Systems, Minneapolis, MN) and IGFBP-2 (DSL, Webster, TX) in serum was determined according to the manufacturer's instructions using ELISA test. Every sample was run in duplicate and the mean was used. The differences between the two measurements were minimal.

Data Analysis and Statistics

All statistical analyses were performed using the R statistical computing environment (vers. 2.12.1). Data for continuous variables are summarized as median with IQ range. Data for categorical variables are presented as frequencies and percentages. Univariate analysis for quantitative variables was based on the non-parametric Mann–Whitney U-test. Chi square test or Fisher exact test was used in case of qualitative variables.

To build a prognostic score able to predict a significant GSU, a multivariate logistic regression modeling was applied using a backward elimination strategy for variable selection. The full model included the preoperative serum levels of PSA, F-PSA, F/T PSA ratio, IL-6, sIL-6R, IGFBP-2, IGFBP-3 together with the clinical stage (dichotomized as T1 or T2) and the biopsy Gleason score (dichotomized as ≤ 5, ≥ 6). At each step, the variable with the highest *P*-values associated with the decrease in -2 log(likelihood) was removed until only effects significantly differing from 0 with $P < 0.05$ were left in the model. The linear predictor of the resulting final model was used as the prognostic risk score.

The performance of the estimated linear predictor was evaluated by computing the area under the receiver operating characteristic (ROC) curve (AUC). Confidence intervals for the AUC and for sensitivities and specificities values were obtained using bootstrapping [36]. Calibration of the fitted model was measured with the Hosmer–Lemeshow statistic [37].

Due to the lack of a test data set, the validity of the prediction model was assessed by a bootstrapping procedure as described in [38] to correct the AUC for over-optimism which occurs when the fit of a model is evaluated using the same data in which the model was built. Two hundred bootstrap samples were drawn with replacement and with the same size as the original sample. The logistic regression model was fitted in the bootstrap sample and the generated scoring system was evaluated, in terms of the AUC metric, in both the bootstrap sample (training set) and in the original sample (test set). The difference between the two AUC, averaged over the 200 bootstrap samples, furnished a stable estimate of the optimism that was subtracted from the apparent performance in order to obtain the optimism-corrected estimate.

In all the analysis, a $P < 0.05$ was considered to be statistically significant.

RESULTS

Table I lists the clinical and pathological characteristics of patients stratified by clinical stage, PSA, % F-PSA, biopsy Gleason score and RP Gleason score.

Significant GSU was observed in 19 patients (36.5%): 14 from ≤ 6 to ≥ 7 and 5 from 7 to 8. The rate of GSU between biopsy and RP in our study population is consistent with several previous reports, where upgrading was present in 32.0% [39], 29.3% [12], and 35.5% [40]of the samples. These data may suggest that our study population is a representative sample for GSU studies. The transition matrix of Gleason score from biopsy to prostatectomy was showed in Table II. The association between the outcome of a significant GSU and all the study variables including the age at serum collection, the serum levels of the analyzed biomarkers, the biopsy T-stage, and the biopsy Gleason score was indicated in Table III. In this univariate analysis, the serum levels of IGFBP3 and T-stage at biopsy were the only variables significantly associated to the presence of GSU. This association was confirmed in the multivariate logistic regression model where also the Gleason score at biopsy emerged as an independent significant predictors of GSU, after adjusting for the other study variables. The coefficients of the final model together with their 95% CI are shown in Table IV. This model produced a risk score able to predict significant GSU. The receiver operating characteristic (ROC) curve describing the prognostic performance of the risk score, showed in Figure 1, indicated a good ability to identify patients who will harbor a higher grade cancer diagnosed at RP [AUC = 0.81 (95% CI 0.69–0.93)]. The optimal cut-off value for the prognostic score maximizing the sum of sensitivity and specificity was -0.42 (corresponding to an estimated probability of GSU of 0.4) with a sensitivity of 0.79 (95% CI 0.58–0.95) and a specificity of 0.82 (95% CI 0.67–0.94). The Hosmer and Lemeshow statistic was 9.4 ($P = 0.309$),

TABLE I. Clinical Characteristics of 52 Patients

Characteristics	Number of patients (%)
Clinical stage	
T1a	1 (2)
T1c	22 (42)
T2a	17 (33)
T2b	2 (9)
T2c	10 (18)
PSA (ng/ml)	
0–4	28 (54)
4.1–10	19 (36)
10.1–20	5 (9)
% F-PSA	
1–10	26 (50)
10.1–15	17 (33)
15.1–20	5 (9)
>20	4 (7)
Biopsy Gleason score	
2–4	3 (5)
5–6	32 (63)
7	17 (31)
Prostate specimen Gleason score	
5–6	24 (46)
7	21 (40)
8–10	7 (13)

TABLE II. Transition Matrix of Gleason Score From Biopsy to Radical Prostatectomy

	Gleason Score at Prostatectomy					
Gleason Score at biopsy	5	6	7	8	9	Total
4	1	0	2	0	0	3
5	2	1	3	0	0	6
6	1	18	9	0	0	28
7	0	2	8	2	3	15
Total	4	21	22	2	3	52

thus showing no evidence for a poor calibration of the fitted model. According to the bootstrapping procedure, the optimism in the AUC was small (0.03) resulting in a optimism-corrected estimate of 0.78 (95% CI 0.67–0.90). The dot-plot of the risk score in all the patients, classified according to the presence or the absence of a significant GSU is showed in Figure 2. The dashed line indicates the optimal cut-off value as derived from the ROC analysis.

DISCUSSION

PSA-based screening has determined detection of early-stage disease at a high cost of overdiagnosis and overtreatment. Consequently, at biopsy there is a need to adequately distinguish between aggressive tumors that require immediate invasive treatment and ''clinically insignificant'' tumors [41]. Several tumor-associated prognostic biomarkers with biological relevance in prostate tumorigenesis or tumor progression have been proposed [17,18]. IGFs family members, IL-6 and its soluble receptor have shown potential as prognostic indicators in PCa [42,43]. Furthermore, recent studies suggested also tertiary Gleason component as prognostic tool at biopsy [44,45]. In this context, we evaluated the risk for PCa patients in our study population to harbor a higher grade cancer as well as the ability of the examined biomarkers to estimate the magnitude of this risk. We showed that low IGFBP-3 levels are good predictor of high-grade cancer diagnosed at RP. Our finding may not be surprising since circulating IGFBP-3 levels previously have been shown to be lowest in patients with bony metastases and lower in patients with metastases to regional lymphnodes than in patients with non-metastatic PCa or in healthy

TABLE III. Descriptive Statistics of Measured Variables in Serum of Patients Stratified by Gleason Upgrade

	Patients without GSU (n = 33, 63%)		Patients with GSU (n = 19, 37%)		
Continous Variables	Median	IQR	Median	IQR	*P* value
Age	65	61–68	64	61–68	0.956
T-PSA, ng/ml	3.84	0.21–17.9	4.06	1.25–16.4	0.812
F-PSA, ng/ml	0.4	0.06–2.18	0.47	0.07–2.43	0.398
F-PSA/T-PSA, %	0.11	0.04–0.33	0.11	0.05–0.27	0.977
IL-6, pg/ml	6.27	2.30–158	6.82	5.4–281	0.408
sIL-6R, ng/ml	61.26	34.22–133.7	70.86	28.62–149.7	0.119
IGFBP-3, μg/ml	3.48	1.48–5.67	2.51	1.9–4.26	0.024
IGFBP-2, ng/ml	812.5	0–1,800	850	200–1,450	0.924
	Patients without GSU		Patients with GSU		
Categorical Variables	N	%	N	%	*P* value
Biopsy stage					
T1	19	57.58	4	21.05	0.011
T2	14	42.42	15	78.95	
Biopsy Gleason Score					
≤5	4	12.12	5	26.32	0.260
≥6	29	87.88	14	73.68	

TABLE IV. Multivariate Logistic Regression Model Coefficients

Variable	Adjusted β-coefficient	Standard error	*P* value	95% CI	
				Lower Bound	Upper Bound
Biopsy stage					
T2 versus T1	1.73	0.76	0.023	0.24	3.22
Biopsy Gleason score					
≥6 versus ≤5	−1.71	0.86	0.048	−3.39	−0.02
IGFBP-3, μg/ml	−0.91	0.46	0.023	−1.80	−0.02
Constant	2.56	1.38	0.064	−0.14	5.26

subjects [46]. Other authors showed that local expression of IGFBP-3 has been inversely associated with Gleason score [25]. These clinical observations agree with biological role of IGFBPs that bind and sequester IGF-1 molecules, suppressing growth under physiologic circumstances [47]. Proteases in plasma and tissues hydrolyze IGFBPs, releasing IGFs and further regulating their action [48]. This kind of regulation is well-rendered in prostate gland, where PSA functions as an IGFBP-3 protease reversing the inhibitory effect of the binding protein on prostate cell growth [49]. Furthermore, it has been reported that IGFBP-3 exerts distinct biological actions such as cell growth inhibition and induction of apoptosis through an IGF/IGF-1 receptor-independent manner in a variety of cancer cells [50–52]. Recently, Ingermann et al. [53] showed IGFBP-3 suppression in breast and PCa. Of note, in androgen-independent PCa progression, androgen-receptor (AR) signaling may be enhanced through activation of AR by growth factors such as IGF and EGF [54].

Previous investigations provided evidence of the relationship between biopsy T-stage and RP Gleason score [7,55]. However, previous authors suggested that single variables may be of limited utility to predict biopsy Gleason score upgrading [39,56,57]. We used logistic regression analysis to evaluate the use of multivariate model and we calculated a risk score including serum IGFBP-3, biopsy T-stage, and biopsy Gleason sum. So, in agree with univariate analysis which indicated a significant association between serum IGFBP-3 (but not serum PSA) and GSU, we combine serum IGFBP-3 in place of serum PSA, as in the currently used model [12], with biopsy T-stage and biopsy Gleason sum. Our data hold with protective role of IGFBP-3 on proliferative potential of

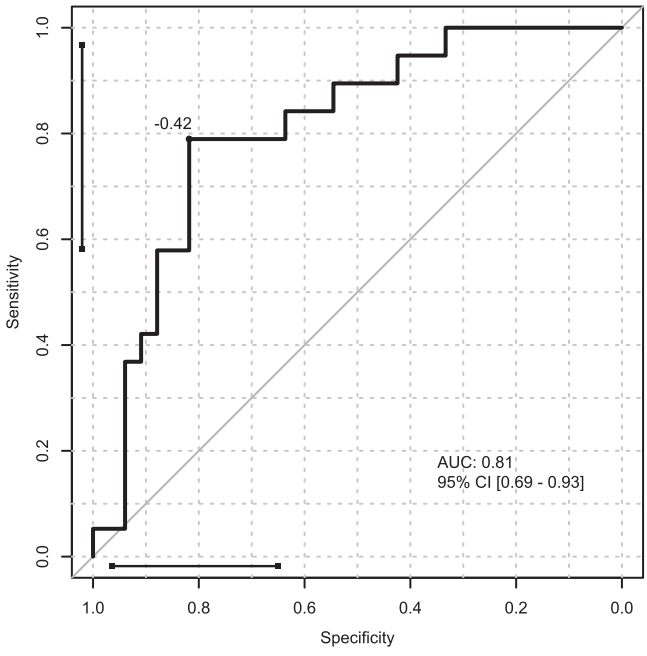

Fig. 1. ROC curve showing risk score ability to predict significant GSU. Vertical and horizontal arrowed lines represent the 95% CI for sensitivity and specificity associated to the optimal cut-off.

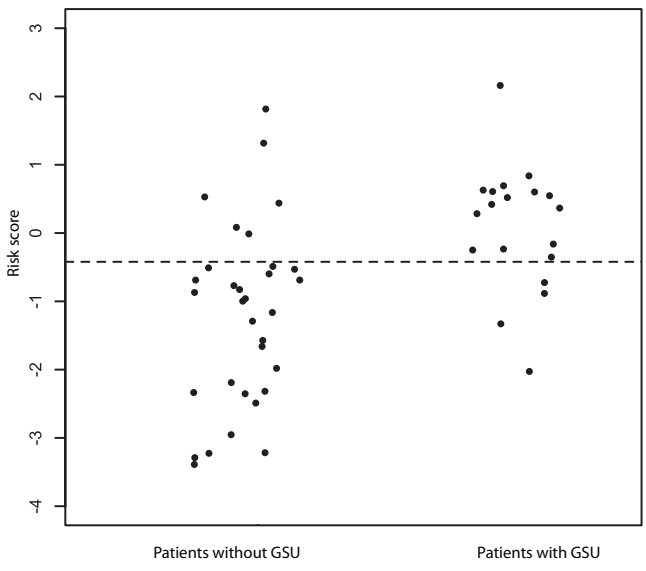

Fig. 2. Dot plot showing risk score in all the patients stratified by significant GSU. The dashed line indicates the optimal cut-off value as derived from the ROC analysis.

cancer cell [48,53] and with not yet clear relationship between PSA and Gleason score.

In conclusion, we found that IGFBP-3 could represent an important tool to predict the risk of patient with biopsy Gleason score ≤ 6 to conceal a higher grade cancer diagnosed at RP. Using a model combining serum IGFBP-3 with biopsy T-stage and biopsy Gleason score, it has been possible in our study population to calculate a risk score with good ability to predict significant GSU. These findings may be useful in clinical management of PCa patients particularly when AS or low dose brachytherapy are considered. Since we showed the ability of risk score to improve the discrimination between patients who were upgraded and those who were not upgraded, we encourage further carefully designed study on large population in order to demonstrate the usefulness of IGFBP-3 as a routine marker for clinical use in PCa patient management, considering also that IGFBP-3 serum values were influenced by genetic variations [58–60].

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