Original Article

Serum biomarkers of inflammation for diagnosis of prostate cancer in patients with nonspecific elevations of serum prostate specific antigen levels

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Background: Lactate dehydrogenase (LDH) and C-reactive protein (CRP) are biomarkers of inflammation commonly used in medicine. The aim was to evaluate the utility of serum LDH and CRP levels for diagnosis of prostate cancer (PC) in men with nonspecific elevations of serum total prostate specific antigen (PSA) levels.

Methods: The following serum biomarkers were measured in patients with PSA between 4 and 10 ng/mL: LDH, CRP and free-PSA. The free-to-total serum PSA ratio (%fPSA) was (free-PSA/PSA) ×100. Patients were classified into two groups according to diagnosis of prostate biopsy: PC and NOT PC patients. Logistic regression was used for develop a probabilistic model to predict PC patients. Diagnostic accuracy was determined using receiver operating characteristic (ROC) curves, calculating the area under the ROC curve (AUC).

Results: We studied 232 patients with ages between 43 and 98 years old (median =72), 200 NOT PC and 32 PC patients. CRP was not statistically significantl to differentiate between PC and NOT PC patients. Probabilistic model (%) was $100\times (1+ e^{-Z})^{-1}$; (Z =0.0070× LDH –0.1589× %fPSA –1.4898). The AUCs were 0.657 (P=0.0048), 0.802 (P<0.0001), and 0.844 (P<0.0001) for serum LDH levels, %fPSA values and probabilistic model, respectively.

Conclusions: CRP was not useful to differentiate benign from malignant prostate disease, in contrast LDH could be used for diagnosis of PC. A probabilistic model using LDH and %fPSA can improve the diagnostic accuracy in patients with PSA between 4 and 10 ng/mL.

Keywords: Biomarkers of inflammation; prostate cancer (PC); prostate specific antigen (PSA); lactate dehydrogenase (LDH); C-reactive protein (CRP)

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Introduction

Prostate cancer (PC) is a major health concern worldwide, being the second most common neoplasm and sixth cause of cancer-related death in the entire world (1). Some studies have found an association between inflammatory biomarkers and PC (2-6).

Serum lactate dehydrogenase (LDH) and C-reactive protein (CRP) are biomarkers of inflammation commonly used in medicine. CRP is an acute phase protein and a very sensitive marker of inflammation and tissue damage.

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It is synthesized mainly in the liver in response to interleukins. Levels of serum CRP can remain elevated in chronic inflammatory processes and cancer (6,7). LDH interconverts pyruvate and lactate at the end of the glycolytic pathway using NAD+ as a cofactor and are present in all tissues. LDH exists in five major isoenzymes, numbered LDH-1 through LDH-5. Serum total LDH is the sum of all the isoenzymes. Concentration of serum LDH can increase in many inflammatory processes and cancer (8).

The gold standard tools currently applied for the diagnosis of PC include the serum total prostate specific antigen (PSA), the digital rectal examination, and the ultrasound-guided systematic prostate biopsy sampling. Serum PSA has become the most clinically useful tumour marker for the diagnosis and subsequent monitoring of PC. The patients are selected for prostate biopsy on the basis of serum PSA levels. Patients with nonspecific elevations of serum PSA levels, values in the intermediate range of 4 to 10 ng/mL, provide less diagnostic certainty, resulting in high false-positive rates and a large number of unnecessary biopsies. The free-to-total serum prostate specific antigen ratio (%fPSA) has been proposed to differentiate benign from malignant prostate disease, improving specificity while maintaining sensitivity, in these patients (9).

The aim of this study was to evaluate the utility of serum LDH and CRP levels for diagnosis of PC in men with serum PSA levels in the intermediate range of 4 to 10 ng/mL.

Methods

This is a prospective and descriptive study whose methodology has been authorized by the Cadiz Ethics of Research Committee and all the participants have signed the informed consent.

Patients

We studied asymptomatic men from Puerto Real University Hospital with no known history of PC and serum PSA levels in the intermediate range of 4 to 10 ng/mL, who underwent 12-core transrectal ultrasound guided prostate biopsy for the first time from 2014 to 2016. Patients with other inflammatory pathologies (autoimmune diseases, hepatitis, pancreatitis, cirrhosis, infectious mononucleosis, sepsis, tumours) or patients with haemolysed samples that could elevate serum biomarkers of inflammation levels were excluded. Patients were classified into two groups according to the diagnosis of prostate biopsy: PC and NOT PC patients.

Biomarkers

Prior to biopsy and after obtaining an informed consent, blood specimens were drawn by venipuncture in gel separator serum tubes and centrifuged at 4,000 rpm for 5 minutes. The measurement of the haemolytic index (HI) were determined by colorimetric method on Hitachi Modular cobas c 702 (Roche Diagnostics, Basel, Switzerland) and just the non-haemolysed samples, those with a HI below 50 units, were included in the study.

The following serum biomarkers were measured: PSA and free-PSA by electrochemiluminescence immunoassay on Hitachi Modular E-170 analyzer (Roche Diagnostics, Basel, Switzerland); LDH by enzymatic photometric method according to the International Federation of Clinical Chemistry and CRP by immunoturbidimetric test with monoclonal anti-CRP antibodies on Hitachi Modular cobas c 702 analyzer (Roche Diagnostics, Basel, Switzerland). The reference range in serum for LDH and CRP is 135–225 U/L and <5.0 mg/L respectively. The %fPSA was calculated using the following formula: (free-PSA/PSA) ×100 (%).

The prostate volume was determined by transrectal ultrasound using the longitudinal and transverse diameters (10): Prostate volume = $[(\text{longitudinal diameter})^2 \times \text{transverse diameter}]/2$.

Statistical analysis

The data obtained was processed by the statistical program Medcalc®, where $P<0.05$ was considered as statistically significant. D'Agostino-Pearson test was used to determine the type variable distribution. Descriptive statistics of the variables with normal distribution were expressed with the range, mean and standard deviation, and variables with non-Gaussian distribution with the range, median and interquartile range. The correlation between variables with normal distribution were analyzed using the Pearson correlation coefficient, and between variables with non-Gaussian distribution using the Spearman rho. The comparison between groups was performed using analysis of variance test for normally distributed variables and Mann-Whitney test for variables with non-Gaussian distribution. Logistic regression was used for develop a probabilistic model to predict patients with PC and determine the importance of each biomarker by calculating the odds ratio. The diagnostic accuracy was determined using receiver

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Table 1 Descriptive statistics of prostate volume and serum PSA, %fPSA, LDH and CRP levels in PC and NOT PC patients

Biomarker	PC	n	Range	Median (95% CI)	IR	P value
Prostate volume (cm^3)	0	200	16.0–96.9	59.0 (33.6–81.0)	44.0	>0.05*
	1	32	14.0–78.5	52.5 (34.7–68.0)	26.5	
PSA (ng/mL)	0	200	4.01–9.95	5.58 (5.19–5.70)	2.89	>0.05*
	1	32	4.05–9.99	6.62 (5.53–7.01)	1.80	
%fPSA (%)	0	200	4.77–58.81	20.46 (16.96–22.56)	14.90	<0.0001*
	1	32	3.80–21.31	8.87 (6.97–13.29)	7.50	
LDH (U/L)	0	200	121–543	206 (195–240)	122	0.0048*
	1	32	138–844	298 (194–449)	272	
CRP (mg/L)	0	200	0.2–363.3	8.2 (6.5–12.5)	35.1	>0.05*
	1	32	0.7–247.2	7.6 (2.9–47.5)	75.0	

*, U Mann-Whitney test. PC, prostate cancer; CI, confidence interval; IR, interquartile range; PSA, serum total prostate specific antigen; %fPSA, free-to-total serum prostate specific antigen ratio; LDH, serum lactate dehydrogenase; CRP, serum C-reactive protein; 0, NOT PC patients; 1, PC patients.

operating characteristic curves (ROC), calculating the area under the ROC curve (AUC) and the optimal cut-off point with its corresponding sensitivity and specificity. The optimal cut-off point was that which had the highest sensitivity and specificity, which correctly classified the largest number of patients.

Results

We studied 232 patients with ages between 43 and 98 years old (median =72), 200 NOT PC patients (86.2%) and 32 PC patients (13.8%). All PC patients had no metastasis, 30 of PC patients showed Gleason score ≤7, and just two with Gleason score =8. All the variables studied followed a non-Gaussian distribution. No statistically significant differences were found between PC and NOT PC patients according to the age, nor was there a significant correlation between the age of the patients and the variables analyzed (P>0.05).

Descriptive statistics of prostate volume and serum PSA, %fPSA, LDH and CRP levels in PC and NOT PC patients are showed in *Table 1*. No statistical correlation was found between prostate volume or %fPSA and LDH or CRP (P>0.05). A low intensity correlation was obtained between LDH and CRP, Spearman rank correlation coefficient (rho) =0.178 (P=0.0068).

In this study, prostate volume, serum PSA and CRP levels were not statistically significantly to differentiate between PC and NOT PC patients (P>0.05). Serum LDH levels and %fPSA values were included in the probabilistic model to predict patients with PC by logistic regression. The odds ratios were 0.8530 [95% confidences interval (CI): 0.7933–0.9173] and 1.0071 (95% CI: 1.0033–1.0108); and coefficients were –0.1589 (P<0.0001) and 0.0070 (P=0.0002) for %fPSA and serum LDH, respectively. The probabilistic model to predict patients with PC was: LDH + %fPSA (probability %) = $100 \times (1+ e^{-Z})^{-1}$; (Z =0.0070 × LDH –0.1589× %fPSA –1.4898).

The ROC curves of probabilistic model, serum LDH levels and %fPSA values to differentiate between PC and NOT PC patients are compared in *Figure 1*. AUC, optimal cut-off value, sensitivity and specificity for the diagnosis of PC using probabilistic model, serum LDH levels and %fPSA values are shown in *Table 2*.

Discussion

In this study, the prostate volume and CRP were slightly higher in NOT PC patients, although they were not useful to differentiate benign from malignant prostate disease in these patients. In contrast, serum LDH levels were significantly higher in the PC patients and %fPSA values were very higher in the NOT PC patients (*Table 1*).

No correlation was found between prostate volume and serum biomarkers of inflammation. There was a very low correlation between serum levels of LDH and CRP (rho =0.178), so they can be considered independent

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biomarkers.

Serum CRP level appears to be an independent prognostic factor of PC (11,12). High concentrations of serum CRP have been associated with shorter overall survival in patients with castration-refractory PCs (13) and with a poor prognosis in PC patients undergoing radiotherapy (14-16), however high serum CRP levels have not been associated with an increased risk of PC (3,6,7,17-22). In this study, serum CRP levels were not useful to differentiate benign from malignant prostate disease in patients with serum PSA levels in the intermediate range of 4 to 10 ng/mL. This result may be due to the fact that serum CRP is a biomarker of inflammation with high sensitivity but low specificity, so NOT PC patients can have elevated serum CRP due to other diseases (including benign prostatic hypertrophy) resulting in a large number of false positives.

Some studies have found an association between LDH and PC: the activity and protein level of mitochondrial LDH isomers (D and L) are higher in tumours cells than in normal cells (23,24); LDH 5 isoenzyme overexpression is significantly linked to highly proliferating prostate carcinomas and with biochemical failure and local relapse following radiotherapy (25); serum LDH levels was suggested to be prognostic indicator in PC patients with bone metastasis (26); and recently we have proposed the combination of serum LDH levels and %fPSA values for the diagnosis of PC using a multivariable score, but a logistic regression analysis was not performed to develop a probabilistic model (27). In this study, serum LDH levels and %fPSA values were independent predictors for diagnosis of PC. Serum LDH levels showed high specificity with low sensitivity and %fPSA values had high sensitivity with low specificity for the diagnosis of PC in men with intermediate serum PSA levels. Probabilistic model to predict patients with PC using serum LDH levels and %fPSA values improved accuracy, exhibiting 93.7% sensitivity and 71.0% specificity. Probabilistic model increased the specificity by 16% compared to using %fPSA alone (*Table 2*). High serum LDH levels in PC patients may be because the tumour cells have high activity of glycolysis, increase glucose consumption and lactate release, requiring higher enzymatic activity of LDH independently from the presence of oxygen (Warburg effect). LDH could be a possible pharmacological target in cancer therapy.

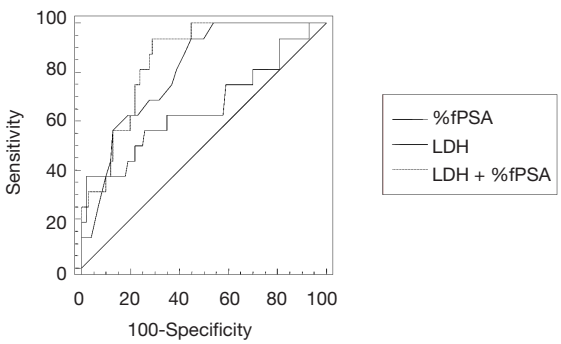

Figure 1 The receiver operating characteristic curves of probabilistic model, serum LDH levels and %fPSA values to differentiate between PC and NOT PC patients. %fPSA, free-to-total serum prostate specific antigen ratio; LDH, serum lactate dehydrogenase; LDH + %fPSA, probabilistic model [probability (%) = 100× $(1+ e^{-Z})^{-1}$; Z =0.0070× LDH –0.1589× %fPSA –1.4898].

In other studies, chronic inflammation of multiple etiologies was a risk factor for PC (6), and serum CRP levels were well-correlated with serum PSA levels in PC patients, suggesting a potential correlation between prostate inflammation and PC (21). In this study, no statistical correlation was found between %fPSA values and serum LDH or CRP levels (P>0.05).

The main limitation of this study is the low number of PC patients (n=32), further studies with larger number of patients are needed to confirm the utility of serum LDH for

Table 2 Area under the ROC curves, cut-off value, sensitivity and specificity of probabilistic model, serum LDH levels and %fPSA values to differentiate between PC and NOT PC patients

Biomarker	AUC (95% CI)	Cut-off	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
%fPSA	0.802 (0.745–0.851) (P<0.0001)	17.42%	93.7 (79.2–99.1)	55.0 (47.8–62.0)
LDH	0.657 (0.592–0.718) (P=0.0048)	436 U/L	37.5 (21.1–56.3)	98.0 (95.0–99.4)
LDH + %fPSA	0.844 (0.797–0.893) (P<0.0001)	13.62%	93.7 (79.2–99.1)	71.0 (64.2–77.2)

ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curves; CI, confidence interval; %fPSA, free-to-total serum prostate specific antigen ratio; LDH, serum lactate dehydrogenase; LDH +% fPSA: probabilistic model [probability (%) =100× $(1+ e^{-Z})^{-1}$; Z =0.0070× LDH –0.1589× %fPSA –1.4898].

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diagnosis of PC.

In conclusion, serum CRP levels were not useful to differentiate benign from malignant prostate disease, in contrast serum LDH levels could be used for diagnosis of PC in patients with serum PSA levels in the intermediate range of 4 to 10 ng/mL. A probabilistic model to predict patients with PC using serum LDH levels and %fPSA values can improve the diagnostic accuracy and reduce the false positive rate, avoiding unnecessary biopsies in patients with nonspecific elevations of serum PSA levels.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.01.31). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This is a prospective and descriptive study whose methodology has been authorized by the Cadiz Ethics of Research Committee and all the participants have signed the informed consent (Prostate cancer 20.10.2016).

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