Ovidius University of Constanta
Doctoral School of Medicine

Field: Medicine

ABSTRACT OF DOCTORAL THESIS

TUMOR BIOMARKERS INVOLVED IN EARLY DETECTION OF PROSTATE CANCER

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CONSTANȚA

2023

This paper "Tumor biomarkers involved in early detection of prostate cancer" proposes to determine new solutions to increase the specificity of detection and progression of one of the most common and expanding tumor pathologies in the male population, prostate cancer.

The international literature contains a small number of articles published in journals, or has a few mentions on this topic in other broader research in the context of more complex underlying themes.

Moreover, this topic, i.e. this type of research, has not been the subject of any other study of this kind in the country.

The study was carried out with the intention of evaluating research on new ways of genetic detection of prostate cancer, and mainly due to new genetic sequencing techniques that evaluate molecular aspects of prostate cancer to highlight the genetic characteristics of the disease.

GST proteins have a complex biology and play multiple roles in cancer cells. These enzymes are a crucial component of the cellular antioxidant system and play critical roles in maintaining cellular homeostasis. Interestingly, recent findings suggest that GST enzymes play an important role in the development of cancer and chemoresistance. However, kinetic and functional studies have shown that most antineoplastic agents are poor substrates of GSTs .

Several studies have shown that GST proteins are overexpressed in many human cancers. Their overexpression contributes to poor outcomes and is negatively correlated with patient survival. However, GSTP1 is not considered a diagnostic marker in clinical practice. We thus suggest that GSTP1, together with a combination of other biomarkers, imaging techniques, minimally invasive surgical techniques, may identify a high-risk population that is susceptible to developing cancer. Active research in the field of antioxidant and redox biology has narrowed in on GSTP1 as a promising therapeutic target for cancer treatment. GSTP1 inhibitors may potentially be used in the future to enhance the efficacy of chemotherapy and overcome drug resistance. However, to use these inhibitors safely for cancer treatment, research is needed to characterise their impact on normal cells and long-term effects.

The main objectives of the project:

1. Assessment of Glutathione-S-Transferase P1 expression as an early diagnostic biomarker and monitoring progression over time;

2. Evaluation of GSTP1 as a primary diagnostic marker; better understanding of angiogenesis in prostate pathology and on mechanisms of tumour angiogenesis;

3. Increase quality of life and decrease mortality through early detection and curative surgery.

Secondary objectives of the project:

1. Analysis of diagnostic and prognostic values of GSTP1 gene expression in tissue samples in differentiating patients diagnosed with CaP and benign prostatic hyperplasia (BPH) by minimally invasive methods.

2. Elimination of repeat biopsy sites in patients with grey-zone PSA levels and/or inconclusive rectal cough.

3. To develop scientific knowledge in the field of Prostate Cancer by promoting and publishing the results obtained.

Patient selection criteria:

The study included a cohort of 80 patients aged 49 to 85 years between 2019-2022 who met the following criteria :

- Age over 18;
- No serious associated pathologies;
- No other known neoplastic pathologies;
- Patients with LUTS symptoms and PSA values within normal limits;
- Patients without LUTS symptoms but PSA values in the grey-zone;
- Patients will sign informed consent prior to any activity related to medical research;

The determination of the gene expression profile of GSTP1 in the whole group was performed by harvesting fresh tissue from both tumour lesions and adjacent normal tissue using

the minimally invasive technique of the Sextant Prostate Biopsy Puncture and consisted of the following activities:

- Immunohistochemical determination of GSTP1 expression in prostate cancer patients
- Postoperative monitoring
- Entering the results into the database
- Statistical analysis of the results obtained
- Interpretation of the results obtained
- Clinical and evolutionary relevance of loss of GSTP1 expression.

Additional parameters monitored following statistical analysis:

Imaging and paraclinical: Transrectal Ultrasound, Nuclear Magnetic Resonance with Prostate Multiparametric Sequencing, Hemoleucogram, serum urea, serum creatinine (whole lot);

Clinical: body mass index, prostate gland volume and consistency by cough-rectal examination (whole lot).

The Ethics Committee for the approval of clinical studies and research works, constituted within the Emergency County Clinical Hospital "Sf. Apostol Andrei" Constanta, by decision nr 446/ 30.03.2018, having analyzed the working protocol, the patient's informed consent form and the retrospective clinical study of the research project "Glutathione-S-transferase gene P1(GST-P1) role in diagnosing prostate cancer in patients with "grey-level" PSA values", favourably endorses the conduct of this study in the Urology Clinical Department, having as principal investigators Conf.Univ.Dr. Felix Voinea and Dr. Marius Doru Stan.

GENERAL ASPECTS

Batch of patients

The patient group comprises 80 cases. These were analysed comparatively using two study groups. These were created on the basis of the diagnosis of certainty obtained from the pathological examination, thus patients were divided into two groups, namely patients with benign tumour (27, 33.8%) and patients with prostate cancer (53, 66.3%).

Table 1 Distribution by positive diagnosis

	Absolute frequency	Percent	Percent Valid	Cumulative Percentage
Benign tumour	27	33.8	33.8	33.8
Prostate cancer	53	66.3	66.3	100.0
Total	80	100.0	100.0	

Age

Regarding their age, we found that patients diagnosed with benign prostate tumor had a mean age of 64.07 years ± 8.9 years, while patients with prostate cancer had an older mean age of 70.02 years ± 8.7 years.

Table 2 Descriptive statistical analysis age by study group

Positive Diagnosis	N	Arithmetic Mean	Standard Deviation	Median	Minimum	Maximum
Benign tumour	27	64.07	8.901	63.00	49	84
Prostate cancer	53	70.02	8.699	70.00	54	85
Total	80	68.01	9.159	68.00	49	85

In terms of their distribution, we found that in patients with benign prostate tumour there is a peak of cases found in the age range 55-59 years (29.6%), with cases also found in patients

under 50 years of age. For patients diagnosed with neoplastic tumour, the distribution did not show a significantly higher frequency for a particular age range, with patients aged 54-85 years.

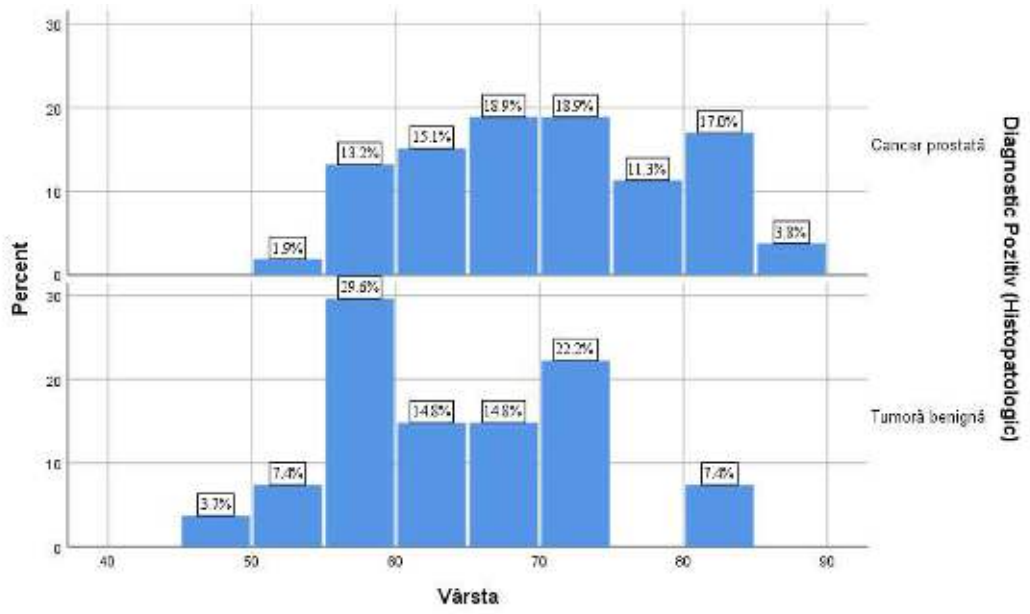

Figure 1 Age distribution by batch

In order to determine the degree to which the distribution of cases differs from a theoretical normal distribution, we used the visual histogram evaluation method as well as the Shapiro-Wilk test. Both the data distributions for the two groups and the Shapiro-Wilk test indicate that the distributions do not differ statistically significantly from a theoretical normal distribution (p=0.33, p=0.095).

Table 3 Normality distribution of age values

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Positive Diagnosis	Statistics	Degrees of freedom	p	Statistics	Degrees of freedom	p
Age	Benign tumour	.123	27	.200*	.958	27	**.330**
	Prostate cancer	.113	53	.087	.962	53	**.095**

*. This is a Limita Inferioară bound of the true significance.

a. Lilliefors Significance Correction

Following the application of the T-test for comparison of age values, the result obtained is statistically significant (p=0.005). Thus, it is found that the observed mean age difference of 5.945 years (95% CI 1.81-10.07 years) is statistically significant, with patients diagnosed with benign tumour being younger.

Table 4 T-test comparing age values by diagnosis

			Age	
			Homogeneous Varieties	Non-homogeneous Varieties
Levene's Test for Equality of Variances	F		.017	
	p		**.896**	
t-Test for Equality of Means	t		-2.868	-2.846
	Degrees of freedom		78	51.377
	P (2 tails)		**.005**	.006
	Average Difference		-5.945	-5.945
	Standard error of the difference		2.073	2.089
	95% confidence interval of the difference	Lower Limit	-10.072	-10.137
		Upper Limit	-1.818	-1.753

Environment of origin

In terms of distribution by background, we found that more than 50% of the patients included in the study came from rural areas. Taking into account that in Constanta County, according to the most recent census data, the proportion of people in urban areas is about 70%, it can be seen that there is a significantly higher proportion of patients coming from rural areas, mainly due to the lack of accessibility to medical information and screening programs in this area.

In terms of the differences observed, depending on the diagnosis, it was found that in the case of patients with benign prostate tumor, about 71% come from rural areas, while in the case

of patients diagnosed with prostate cancer, the proportion of those from rural areas is significantly lower, 41.5% (*Table 5*).

Table 5 Distribution of patients by diagnosis and background

			Positive Diagnosis		
			Benign tumour	Prostate cancer	Total
Environment of origin	Urban	Number	8	31	39
		% of Positive Diagnosis	29.6%	58.5%	48.8%
	Rural	Number	19	22	41
		% of Positive Diagnosis	70.4%	41.5%	51.2%
Total		Number	27	53	80
		% of Positive Diagnosis	100.0%	100.0%	100.0%

Thus, there is a statistically significant association between diagnosis and background (p=0.015).

Table 6 Chi-square test for association between diagnosis and background

	Value	Degrees of freedom	P (2 tails)	P exactly (2 tails)	Exact P (1 tail)
Chi-square	5.964[a]	1	**.015**		
Yates correction[b]	4.864	1	.027		
Likelihood ratio	6.100	1	.014		
Fisher Exact Test				.019	.013
Mantel-Haenszel test	5.889	1	.015		
Number of valid cases	80				

a. 0 cells (0.0%) have expected values less than 5. The minimum expected value is 13.16.
b. Calculated for 2x2 table only

After calculating the Odds Ratio (OR), the result indicates a significantly lower probability 0.299 (IÎ95% 0.111-0.804) that an urban patient will be diagnosed with benign prostate tumor compared to rural patients (Table 7).

Table 7 Odds ratios - estimating the risk of benign prostate tumour diagnosis in urban patients

	Value	95% disbelief	
		Lower Limit	Upper Limit

Quota ratio Environment of origin (Urban / Rural)	.299	.111	.804
Number of valid cases	80		

Relation of background - Age

Descriptive analysis of age by background and type of diagnosis revealed that the mean age of rural patients diagnosed with TB was significantly lower than the other categories, being 61.89 years. In the other situations, the average age was around 70 years, as can be seen in *Table 8*.

Table 8 Age descriptive analysis by background and type of diagnosis

Environment of origin	Positive Diagnosis	N	Arithmetic Mean	Standard Deviation	Median	Minimum	Maximum
Urban	Benign tumour	8	69.25	8.664	72.00	56	83
	Prostate cancer	31	69.58	8.713	68.00	55	85
	Total	39	69.51	8.590	70.00	55	85
Rural	Benign tumour	19	61.89	8.266	59.00	49	84
	Prostate cancer	22	70.64	8.845	71.00	54	85
	Total	41	66.59	9.555	65.00	49	85
Total	Benign tumour	27	64.07	8.901	63.00	49	84
	Prostate cancer	53	70.02	8.699	70.00	54	85
	Total	80	68.01	9.159	68.00	49	85

In terms of how patients are distributed by age, it can be seen that for people with prostate cancer they show a relatively even distribution, with values ranging from 49 to 85 years.

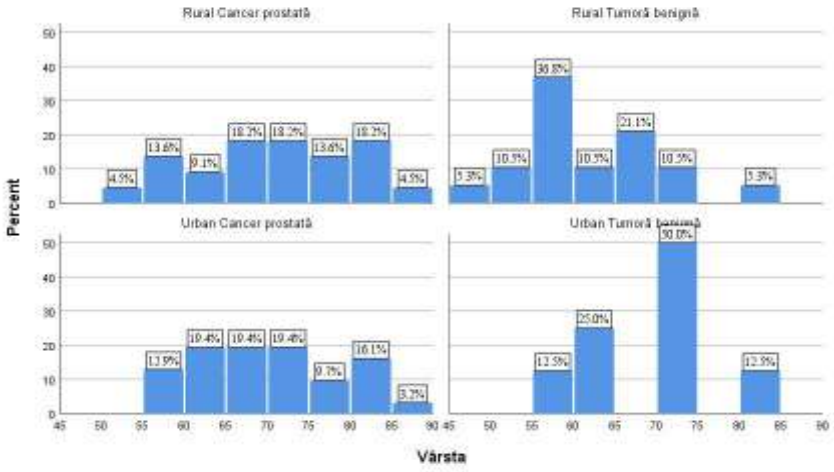

Figure 2 Age distribution by background and diagnosis

In the case of patients with benign prostate tumours, we observed, within the age distribution, that for rural areas there is a preponderance of patients aged 55-60 years, representing more than 36% of cases of benign tumours diagnosed in rural patients, while for urban areas, 50% of patients diagnosed with benign prostate tumours were aged 70-75 years.

However, the result of ANOVA test, the differences observed between groups are statistically insignificant, p=0.154.

Table 9 ANOVA test for testing age differences between groups

		Sum of squares	Degrees of freedom	Mean squares	F	p
Intergroups	(Combined)	171.293	1	171.293	2.070	**.154**
Intragroup		6455.695	78	82.765		
Total		6626.987	79			

Role of Glutathione-S-transferase in the diagnosis of prostate cancer

GST-P1 reactivity

We assessed GST-P1 reactivity in patients included in the study. Following their classification, we found that more than 80% of patients histopathologically diagnosed with benign prostate tumor showed negative values for GST-P1 reactivity. In comparison, in patients diagnosed with prostate cancer, the percentage of those with lack of GST-P1 expression was approximately 30% (*Table 10*).

Index test (methylation status GST-P1)

The index assay (methylation status of GST-P1) can be methylated or unmethylated. The methylation-specific PCR reaction for GST-P1 was performed using the WIZ GSTpi CpG Amplification Kit (Merck KGaA) according to the manufacturer's instructions. In terms of protocol, primer set U was defined as the primer set that annealed to unmethylated DNA that underwent chemical modification, primer set M was the primer set that annealed to methylated DNA, and primer set W was the primer set that served as a control for the efficiency of chemical modification. The primer sequence was not provided by the manufacturer, who stated only that the amplified region is defined as the sequence between the 3' nucleotide of the sense primer and the 3' nucleotide complement of the antisense primer for each gene promoter. The nucleotide numbering system was the one used in the GenBank submission, identified as AY324387 for

GSTpi. For each experiment, controls provided by the assay were used, namely U control DNA and M control DNA, which were amplified with the appropriate primer set and served as controls for unmethylated and methylated DNA, respectively, and untreated W genomic control DNA, which was amplified with the W primer set and served as a control for chemical modification efficiency. PCR products were electrophoresed on a 2% agarose gel and visualized with ethidium bromide. Finally, a negative PCR control (i.e. no DNA) was performed for each primer set.

Test specificity and sensitivity were determined to obtain positive and negative predictive values of the test. Confidence intervals (CI) of 95% were calculated to quantify the statistical precision of the measurements. For comparison of continuous variables, mean and standard deviation (mean ± SD) are presented, and comparisons were performed using Student's t-test for independent variables. For comparisons of proportions, the $\chi 2$ test was used for dichotomous variables.

Summary data for these variables are presented as proportions. To determine the relationship between PSA values and GST-P1 methylation status, a point-biserial correlation was used. This method is a special case of Pearson's product moment correlation applied to a dichotomous variable and a continuous variable, as described in the IBM documentation for SPSS (v.19.0). It was considered that $P<0.05$ indicates a statistically significant difference. The study was approved by the Ethics Committee (no. 446/30.03.2018) of the Clinical Research Ethics Committee (no. 446/30.03.2018) Clinical Studies of the Hospital Clinic Județean de Urgență de Constanța. The procedures in all phases of the study were conducted in accordance with the principles of the Declaration of Helsinki. Informed consent forms were received from all participants prior to enrolment in the study group. (221)

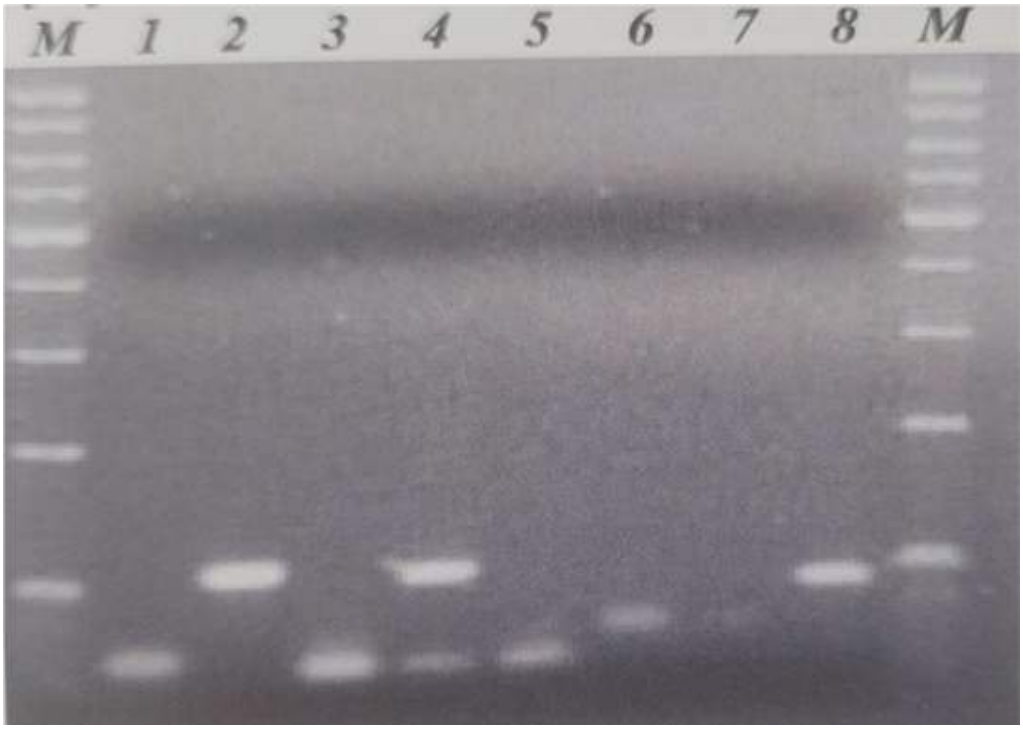

***Figure 12 - Specificity of amplification with** GSTpi*

2% agarose gel analysis using primers and control DNA samples are as follows:

Row 1: W primers, NTC

Row 2: primers W, DNA W

Row 3: U primers, NTC

Row 4: primers U, DNA U

Row 5: primers U, DNA M

Row 6: M, NTC primers

Row 7: primers M, DNA U

Row 8: primers M, DNA M

Row M: marker spaced at 100bp

Above you can see some results obtained with the electron microscope during data processing, as follows:

The WIZ GSTpi CpG Amplification Kit contains primers that can be used for methylation-specific PCR analysis of DNA after prior treatment with the EpiTect Bisulfite kit (Qiagen), which causes changes between methylated and unmethylated DNA. Primer sets in the kit are specially synthesised to analyse DNA for sequence differences.

The set of U primers will amplify the unmethylated DNA that followed the chemical modification.

The M primer set will amplify the methylated DNA that followed the chemical modification.

The set of W primers serve as a control of the chemical modification efficiency. It will amplify any DNA (unmethylated or methylated) that has not undergone chemical modification, i.e. 'wild type' or W. Interpretation of the data can be performed even in the case of incomplete chemical modification (up to 50%).

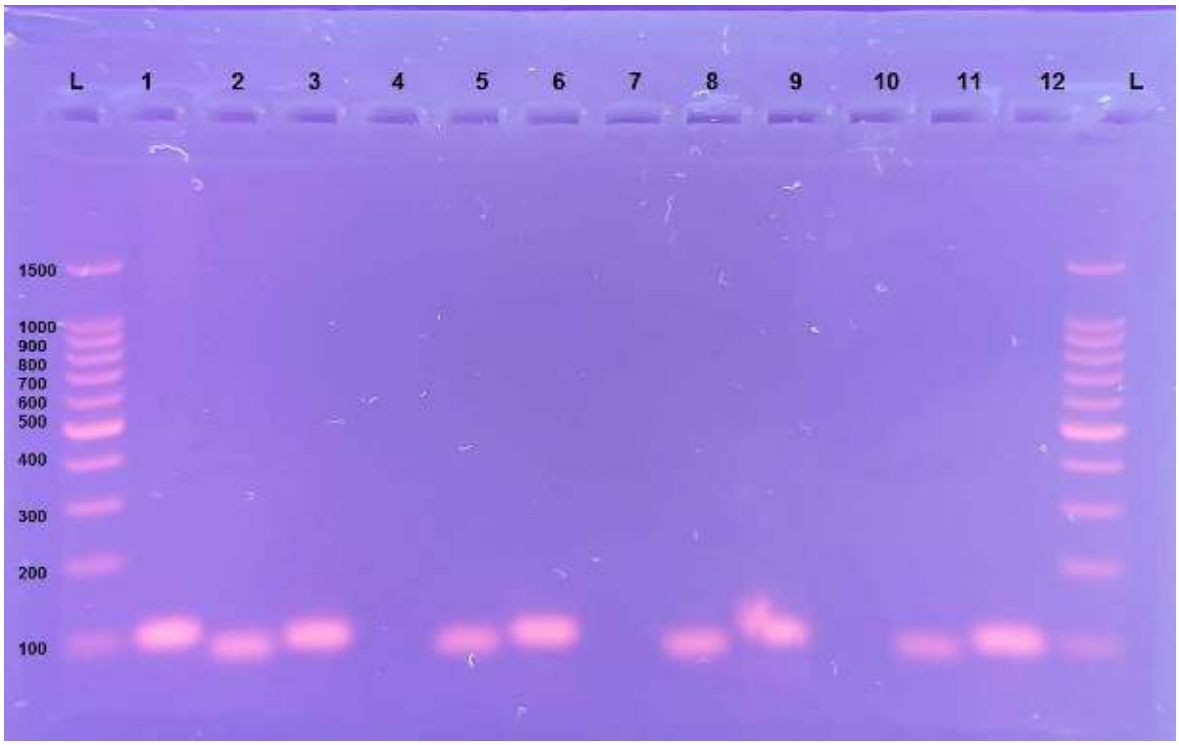

Figure 13 - 2% agarose gel analysis of PCR specific for GSTP1 methylation. *L band: 100bp Ladder DNA. Lane 1: Wylde-type primers with Wylde-type DNA control, Lane 2: Unmethylated primers with unmethylated DNA control, Lane 3: Methylated primers with methylated DNA control. Lanes 4-6: Experimental sample 1 with Wylde-type, unmethylated and methylated primers, Lanes 7-9: Experimental sample 2 with Wylde-type, unmethylated and methylated primers, Lanes 10-12: Experimental sample 3 with Wylde-type, unmethylated and methylated primers.*

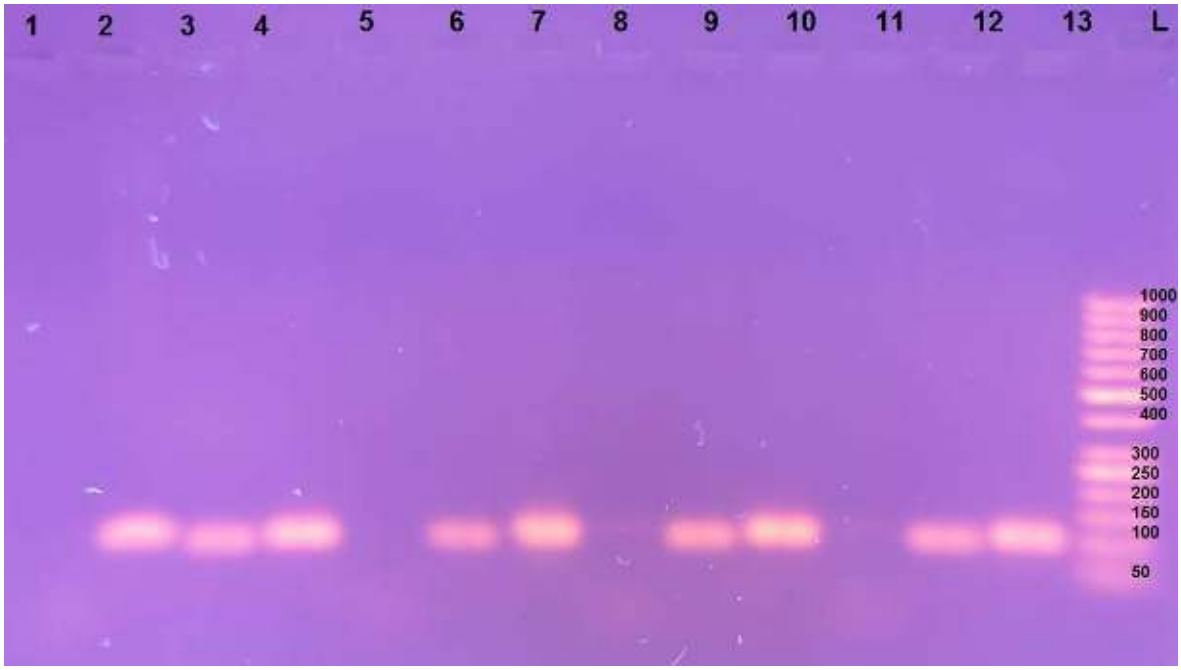

Figure 14 - 2% agarose gel analysis of specific PCR for GSTP1 methylation.

Lane 2: Wylde-type primers with Wylde-type DNA control, Lane 3: Unmethylated primers with unmethylated DNA control, Lane 4: Methylated primers with methylated DNA control. Lanes 5-7: Experimental Sample 4 with Wylde-type unmethylated and methylated primers, lanes 8-10: Experimental Sample 5 with Wylde-type unmethylated and methylated primers, lanes 11-13: Experimental Sample 6 with Wylde-type unmethylated and methylated primers, lane L: 50-100bp Ladder DNA.

The observed difference is statistically significant ($p<0.001$, *Table 11*), indicating a statistically significant association between GST-P1 reactivity and prostate cancer diagnosis, for patients whose PSA values fell in the "grey zone".

Table 10 Distribution of patients according to histopathological diagnosis and GST-P1 reactivity

			Positive Diagnosis		
			Benign tumour	Prostate cancer	Total
GST-P1 reactivity	Negative	Number	22	16	38
		% of Positive Diagnosis	81.5%	30.2%	47.5%
	Positive	Number	5	37	42
		% of Positive Diagnosis	18.5%	69.8%	52.5%
Total		Number	27	53	80
		% of Positive Diagnosis	100.0%	100.0%	100.0%

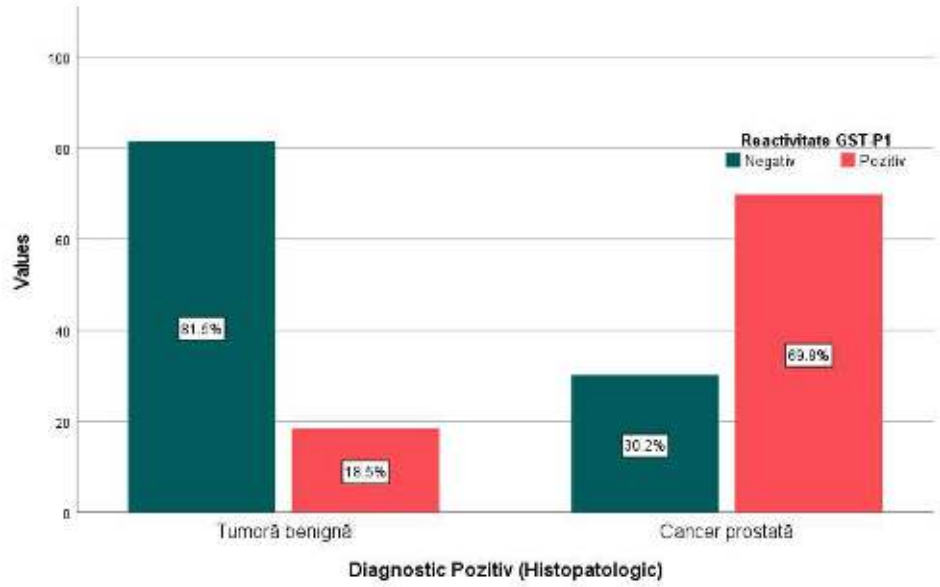

Figure 3 Distribution of patients by diagnosis and GST-P1 reactivity

Table 11 Chi-square test for testing the association between diagnosis and GST-P1 reactivity

	Value	Degrees of freedom	P (2 tails)	Exact P (2 tails)	Exact P (1 tail)
Chi-square	18.872[a]	1	**<.001**		
Yates correction[b]	16.871	1	<.001		
Likelihood ratio	19.908	1	<.001		
Fisher Exact Test				<.001	<.001
Mantel-HaenszelAssociation test	18.636	1	<.001		
Number of valid cases	80				

a. 0 cells (0.0%) have expected values less than 5. The minimum expected value is 12.83.
b. Calculated for 2x2 table only

Thus, the chance of a patient with GST-P1 reactivity being diagnosed with prostate cancer is 10.175 times higher (IÎ95% 3.27-31.64) (*Table 12*)

Table 12 Estimated risk of prostate cancer diagnosis

		Range 95% Confidence	
	Value	Lower Limit	Upper Limit

GST-P1 reactivity ratio (Negative / Positive)	10.175	3.272	31.637
Number of valid cases	80		

Role of GST-P1 in prostate cancer diagnosis

The specificity for the diagnosis of prostate cancer in patients with PSA values in the range of 4-10 ng/ml was 69.81% (95% CI 55.66%-81.66%) and the specificity was 81.48% (95% CI 61.92%-93.7%) (*Table 13*)

Table 13 Analysis of values for diagnostic test - GST-P1

Sensitivity	69.81%	55.66% to 81.66%
Specificity	81.48%	61.92% to 93.70%
Area Under the ROC Curve	0.76	0.65 to 0.85
Positive probability rate	3.77	1.68 to 8.48
Negative probability rate	0.37	0.24 to 0.58
Prevalence of the disease	66.25%	54.81% to 76.45%
Positive probability rate	88.10%	74.37% to 96.02%
Negative predictive value	57.89%	40.82% to 73.69%

At the same time, based on the prevalence calculated in the study, the positive predictive value was 88.1% (IÎ95% 74.37% - 96.02%), and the negative predictive value had lower values of 57.89% (IÎ95% 40.82% - 73.69%).

ROC curve was performed for GST-P1 for prostate cancer diagnosis. Thus, the calculated area was 0.756 (*Table 14*), with 95% confidence interval 0.648 - 0.846, statistically significant $p<0.001$.

Table 14 Area under the ROC curve

Area Under the ROC Curve	0.756
Standard Error [a]	0.0496

Range 95% confidence[b]	0.648 to 0.846
z statistic	5.167
Statistical significance level (p)(Area=0.5)	**<0.0001**

[a] DeLong et al., 1988
[b] Binomial exact

Figure 4 shows the ROC curve. The blue area represents the confidence interval for this.

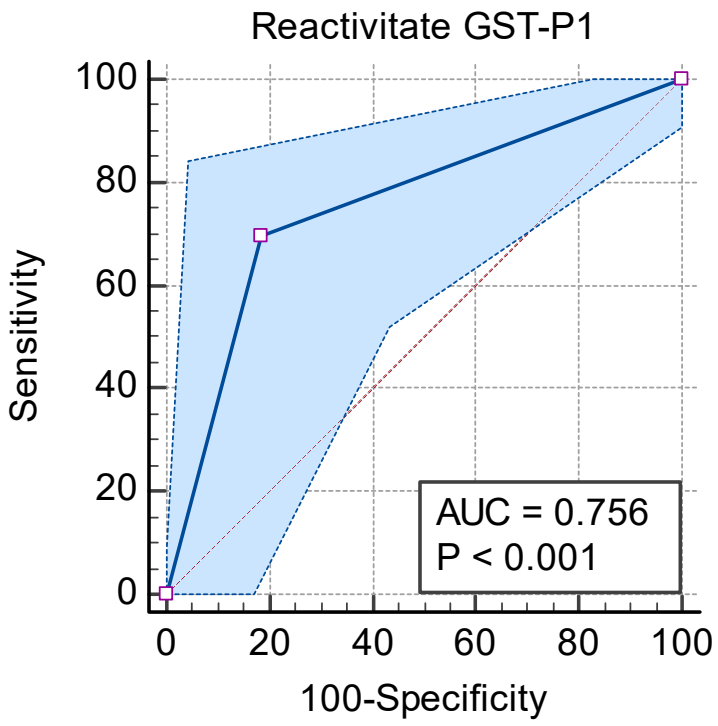

Figure 4 ROC curve for GST-P1 reactivity and prostate cancer diagnosis in patients with inconclusive PSA values

The analysis aimed to assess the potential to use the GST-P1 gene as a biomarker for prostate cancer diagnosis in patients for whom PSA values are inconclusive - in the 'grey' range, defined as values in the range 4-10 ng/ml.

The result indicates that GST-P1 has a high potential to identify patients with prostate cancer. The calculated sensitivity was 69.81%, while the specificity was 81.48%, with a positive probability rate of 88.1% and negative predictive value of 57.89%. These results suggest that GST-P1 assessment, in patients for whom PSA is inconclusive, may provide new information in

the process of diagnosing the presence of prostate cancer, thus aiding earlier detection and initiation of treatment, with subsequent improvement in survival.

PSA - GST-P1 relationship

We further examined the existence of differences between patients with positive and negative GST-P1 reactivity and mean PSA values, respectively. Descriptive statistical analysis is reported in *Table 15*. It is observed that the mean values, standard deviation as well as the median are close in value.

Table 15 Descriptive statistical analysis of PSA by GST-P1 reactivity

PSA value (ng/ml)						
GST-P1 reactivity	N	Arithmetic Average	Standard Deviation	Median	Minimum	Maximum
Negative	38	6.943	1.817	6.53	4.30	10.00
Positive	42	7.238	1.839	6.975	4.10	10.00
Total	80	7.098	1.823	6.80	4.10	10.00

In terms of how they are distributed, it can also be seen in

Figure 5 that no significant differences were detected between the two groups of patients.

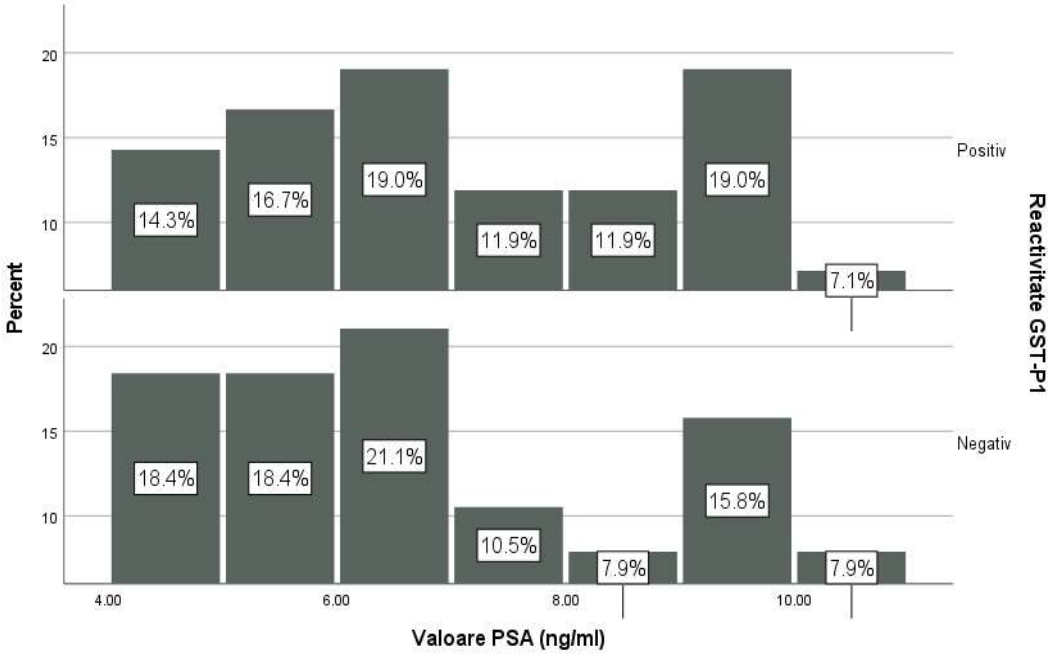

Figure 5 Distribution of patients according to PSA values and GST-P1 reactivity

Based on histogram analysis (

Figure 5), and Shapiro-Wilk test values (*Table 16*), whose result is statistically significant, for comparison we used the Mann-Whitney U test. Its result, in which, the mean rank is higher for patients with positive reactivity (42.89) compared to those with negative reactivity (38.53) (

Table 17). Differences are statistically insignificant, z=-0.723, p=0.47 (*Table 18*)

Table 16 Normality test distribution of PSA values according to GST-P1 reactivity

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	GST-P1 reactivity	Statistics	Degrees of freedom	p	Statistics	Degrees of freedom	p
PSA value (ng/ml)	Negative	.130	38	.104	.915	38	**.007**
	Positive	.117	42	.166	.934	42	**.018**

Table 17 Analysis of PSA ranks according to GST-P1 reactivity

	GST-P1 reactivity	N	Medium Rank	Sum of ranks
PSA value (ng/ml)	Negative	38	38.53	1464.00
	Positive	42	42.29	1776.00
	Total	80		

Table 18 Mann-Whintey U test for testing statistical significance of observed differences in PSA values according to GST-P1 reactivity

	PSA value (ng/ml)
Mann-Whitney U	723.000
Wilcoxon W	1464.000
Z	-.723
P (2 tails)	**.470**
a. Independent variable: GST-P1 reactivity	

DISCUSSIONS

GST-P1 methylation is the most common genetic alteration identified in prostate cancer (223). The results of the analysis indicated that GST-P1 has a potential to differentiate patients with benign prostate tumour from those with prostate cancer where PSA values are inconclusive. The calculated sensitivity was 69.81% and the specificity of the test was 81.48%. The positive predictive value was 88.1% and the negative predictive value was 57.89%. These results suggest that GST-P1 assessment may provide new information in the process of diagnosing the presence or absence of prostate cancer, allowing earlier detection and earlier initiation of treatment.

Methylation of the GST-P1 gene is the most common genetic alteration that has been reported in prostate cancer (201, 202), being observed in more than 90% of prostate cancers, and is rarely seen in benign tumor tissue (203). A recent systematic review and meta-analysis (204) estimated the incidence of GST-P1 methylation higher in prostate cancer patients, with an odds ratio OR=18.58, iÎ95 9.6 - 35.35, $p<0.001$. GST-P1 detection has been investigated in several studies as a potential non-invasive diagnostic tool for early detection of prostate cancer (205, 206) and was also evaluated in a meta-analysis (207). The results show a high variability as concluded by Wu (207), with excellent specificity of GST-P1 (89%, IÎ95% 80%-90%) and lower sensitivity of 63% (iÎ95% 50%-75%).

Another meta-analysis comprising 35 studies aimed to assess the usefulness of GST-P1 in prostate cancer diagnosis (208) and concluded that the sensitivity for GST-P1 (from biopsies) was 81.7% ± 8.3 and the specificity was 95.8% ± 0.6.

Other recent studies have suggested that GST-P1 may be involved in the development and progression of various cancers, with its role in lung, colorectal, gastric and even metabolic cancers having been evaluated in recent research (209).

While previous research has generally had participants screened for the presence of prostate cancer (so the test characteristics were applied population-wide), the particularity of this study is that the participants are patients for whom PSA values were inconclusive (ranging from 4 - 10 ng/ml). This inclusion criterion may be an explanation for the lower specificity values

when compared to those obtained in other studies and may also explain the higher sensitivity value.

Another major difference, which given the purpose of a screening test could be a limitation, relates to the method of measuring GST-P1 methylation status, which was performed by genomic isolation of DNA from biopsy tissue. Previous studies (206, 210-212) have indicated that there is a correlation between the detection of GST-P1 in tissue samples and the methylation status examined in urine samples within certain limits. Other studies have shown significant differences in the sensitivity and specificity of GST-P1 for prostate cancer identification, depending on the method used for testing (213).

The use of genetic markers for the diagnosis of cancer diseases is increasingly common and their potential is very high. The results from this study have the potential to support the use of these diagnostic methods in patients with suspected prostate cancer, but the PSA values are inconclusive. When the PI-RADS score was used in parallel, we observed that diagnostic accuracy increased.

A major role in the survival of cancer patients is played by the medical system's ability to diagnose as quickly as possible. For prostate cancer, prostate-specific antigen and rectal cough are widely used and are recognised as methods used in the diagnosis of prostate cancer (214) and are relatively easy to perform and at low cost to the patient. The use of rectal cough as a predictor of prostate cancer is useful in symptomatic patients (215), it can be used as a first method of investigation. An abnormal result is an indicator of prostate cancer risk, thus leading to more specialised investigations for diagnostic purposes. At the same time, PSA can have a large number of insignificant results, with low sensitivity (216) when the 4ng/ml limit is used, and with a significant number of inconclusive results. These cases require further investigation to clarify the diagnosis (217), thus recent research suggests that PSA testing should be evaluated and discussed with patients (218) to maximize the benefits and limit the undesirable effects this procedure may have.

From the analyses performed, we observed that PI-RADS values of at least 4 provide a high specificity for prostate cancer diagnosis of 39.6%. Such a result offers very good prospects to be used to identify patients without prostate cancer and with borderline PSA values. These

results are similar to those reported in the literature (219, 220, 221) when PI-RADS was used for prostate cancer diagnosis.

When the score was combined with GST-P1 testing, the accuracy of the diagnostic imaging method increased statistically significantly ($p=0.014$). The results suggest that by combining different patient assessment methods, the success rate for a correct and rapid diagnosis increases significantly. Assessment by PI-RADS and GST-P1 methylation in patients with inconclusive PSA values provides better specificity and sensitivity compared to testing performed separately. The use of these procedures can improve the diagnostic process by identifying prostate cancers with greater accuracy. These results support the potential to improve the diagnostic process for prostate cancer.

CONCLUSIONS

The total number of patients was 80. As the study was retrospective, tests were performed on all patients without dropout. The main feature of the sample was that all participants had PSA values between 4 and 10 ng/ml.

Patients diagnosed with prostate adenocarcinoma tend to be older in age (70.02 years; SD= 8.7) compared to patients diagnosed with prostate adenoma (64.07 years; SD= 8.9), and these patients are predominantly from urban areas.

All parameters analyzed in the remainder, i.e. prostate volume, irritative lower urinary symptoms, PSA values, did not show statistically significant differences (all P-values >= 0.5).

Digital rectal examination raised the suspicion of prostate cancer in 69.8% of patients diagnosed with adenocarcinoma, but at the same time, suspicion of malignancy was also suggested in 29.6% of cases diagnosed with prostate adenoma.

A point-biserial correlation analysis was performed to determine the relationship between PSA values and GST-P1 methylation status. A positive correlation was identified, although this was not found to be statistically significant (rpb=0.081; n=80; p=0.473)

In addition, more detailed attention was paid to the results of GST-P1 reactivity in patients in the grey zone of PSA values. Of the 53 patients diagnosed with PCa, 69.8% (n=37) were GST-P1-positive, while of the 27 patients diagnosed with BPH, 18.5% (n=5) were GST-P1-positive. The calculated accuracy of the test was 73.75%, as it correctly identified 37 patients with PCa and 22 patients with BPH.

The calculated sensitivity for diagnosing PC in patients with PSA values between 4 and 10 ng/ml was 69.81% (95% CI, 55.66-81.66%), and the specificity was 81.48% (95% CI, 61.92-93.70%) (Table II). At the same time, based on the prevalence given by the study population, the positive predictive value was determined to be 88.1% (95% CI, 74.37-96.02%), and the negative predictive value had a lower value of 57.89% (95% CI, 40.82-73.69%). The receiver operating characteristic (ROC) curve was subsequently plotted for GST-P1 and PSA for PCa diagnosis.

PI-RADS lesions and GST-P1 methylation testing when PSA levels are in a 'grey zone', offer better specificity and sensitivity compared to single testing. Testing patients with inconclusive PSA levels, allows for more accurate diagnosis and less overdiagnosis by non-

invasive procedures such as repeat biopsies. These results further support the potential for improved diagnosis through interleaved imaging studies and prostate biomarkers.

When combined ă with GST-P1 testing, the accuracy by imaging method of prostate cancer diagnosis increased in a statistically significant way ($p=0.014$). The results in this study suggest that by combining different methods of patient assessment, the success or success rate of an accurate and timely diagnosis is significantly improved, thus contributing also to oncological staging and therapy after diagnosis.

However, there is a need for new prognostic and/or diagnostic biomarkers that allow more accurate stratification, not only of PCa risk, but also of clinical relapse risk or monitoring of tumour progression. Some of the biomarkers studied already have commercially available tests, others are in the process of validation, and others require validation in data sets or in patients with large independent sample sizes before clinical use.

The association between GSTP and cancer was encouraged by the fact that overexpression of GSTP has been observed in many chemotherapy-resistant cancers. However, functionally, it was realized that most anticancer drugs are weak substrates for GSTP1, with a weaker catalytic constant for GSTP1 conjugation reactions, therefore, attention was directed towards the involvement of GSTP in several cellular functions, particularly in the regulation of various kinases and the post-translational S-glutathionylation process of several proteins.

Research in this area is particularly active and promises to define specific GSTs and their role in cancer, or specific polymorphic forms of GSTs as possible pharmacological targets.

Thus, new methodological approaches will enable physicians to target their efforts more effectively, more precisely to identify true prognostic risk, guide personalized management to help control the disease, and improve survival and quality of life. Prostate cancer is one of the most important pathologies in oncology. There is no ideal therapy for any of its stages, and even today, many patients suffer from the disease itself or the side effects of treatment.

Molecular biology encompasses different types of research, such as genomics, proteomics, epigenetics and phage, which may in the near future reveal specific details of disease initiation and progression.

Scientists are constantly looking for better ways to diagnose PCa, to predict which patients will have recurrence after initial treatment and to establish better markers of disease onset, progression and prognosis.

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