EFFICIENCY OF PROSTATE BIOPSIES STRUCTURED IN MULTIPLEX FORMAT IN HISTOPATHOLOGICAL STAGING OF PROSTATE CANCER

I. Introduction

Over the past decade, modern medicine has made significant progress, but prostate cancer (PC) continues to be a public health problem, with a steadily increasing prevalence among the male population. PC is also one of the most common cancers in men and is one of the significant causes of death among men.

The widespread implementation of prostate biopsy and screening performed by serum prostate-specific antigen (PSA) testing had a formidable result in increasing early diagnosis of PC, as well as significantly decreasing cases of metastatic PC at the time of diagnosis. Taking into account the long evolution of PC, the benefits of screening become more evident as the monitoring period expands. An update to the ERSPC study, published in 2019, revealed a decrease in the number of cases needed to diagnose and thus prevent death by PC[1]. In a meta-analysis of four randomized trials involving 675,232 people, men who were screened were found to have a higher incidence of cancer diagnosis (incidence rate ratio 1.23, with a confidence interval 95% 1.03-1.48) compared to the control group. [2] Despite the growing trend of early detection of PC, extensive screening for PC has become a controversial topic in the urological medical world today.

A key role in the diagnosis, prognosis and management of PC is played by tumor biomarkers. The research trend is on identifying non-invasive tumor biomarkers by using modern technologies to support doctors in making difficult clinical decisions. I believe it is crucial to develop new markers and biomarkers for the diagnosis, prognosis and monitoring of treatment in both non-metastatic and metastatic prostate cancer, as well as to implement a personalized treatment strategy for proper management of each stage of PC. The risk factors that contribute to the development of PC are not fully clear, but age and genetic factors play an important role in the risk and progression of this disease. Currently, preoperative PSA serum levels, tumor stage and Gleason histological grade are the only prognostic factors used in clinical practice for newly diagnosed PC patients. In current medical practice, the use of the Gleason

score is essential for the treatment decision in prostate cancer. The Gleason classification system for prostate cancer is the most widely used method worldwide in both research and clinical practice.

The Gleason score is based exclusively on the architectural characteristics of prostate cancer cells and closely correlates with clinical behavior. A higher score indicates a higher probability of having a disease extended outside the organ, as well as a weaker result after treatment of localized disease [3.4]. Based on the growth pattern and degree of differentiation, tumors are classified from 1 to 5, grade 1 being the most and grade 5 the least differentiated. [5].

The Gleason score was the preferred system for tumor grading and was incorporated as a key prognostic factor in the 2010 tumor, nodules, metastasis (TNM) staging system for prostate cancer. In the eighth edition (2017) of the TNM staging system, information about the Gleason score was incorporated into the new histological grade group, which is used to assign patients to prognostic stage groups. The consensus Conference of the International Society of Urological Pathology (ISUP) in 2014 adopted a new five-level classification system based on modified Gleason scores [6]. This new classification system (ISUP grade group), which has been updated [7], is used in the new 2022 World Health Organization (WHO) classification of prostate tumors [8]. The new grade group system is not designed to replace the Gleason classification system; Instead, it is based on the Gleason score and provides a more accurate risk classification than the compound Gleason score [9]. Tumors are divided into five categories based on the primary and secondary Gleason model. The grade group system was validated in an analysis of more than 20,000 patients undergoing radical prostatectomy at five academic centers between 2005 and 2014 [10.11]. In the validation study, there was an increased risk of mortality from prostate cancer with the increase of the overall grade group [12]: ISUP 1: Gleason score ≤6; ISUP 2: Gleason score 3+4 = 7 (risk ratio [HR] for death 2.8 compared to grade group 1); ISUP 3: Gleason score 4+3 = 7 (HR 6.0 relative to grade group 1); ISUP 4: scor Gleason = 8 (including 4+4 = 8, 3+5 = 8 or 5+3 = 8; HR 7.1 in relation to group note 1); ISUP 5: Gleason scores from 9 to 10 (4+5, 5+4 or 5+5; HR 12.7 compared to grade 1). In another report, the five-year recurrence-free survival rates were 95, 83, 65, 63 and, respectively, 35 %, for grade group 1, 2, 3, 4 and 5 [12]. In 1966, Donald Gleason published the Gleason score in Cancer Chemotherapy Reports, which was initially performed on 270 patients and later validated on a study of 4000 patients. Later, researcher Akhouri Sinha for four decades described the Gleason scoring system as “comprehensive, but simple, so that the classification system can be used by pathologists, clinicians and scientists around the world.” [13] The score was adopted gradually until 1987,

when several leading experts in the field recommended its use in all scientific publications on PC. The Gleason score became even more used following an increase in prostate cancers identified by a blood test, the prostate-specific antigen (PSA) test. In 2009, according to Dr. Bruce Roth, a professor at Vanderbilt University, said, "every prostate cancer patient knows their Gleason score." Roth noted that it was remarkable that the Gleason score remained the gold standard in the profession, despite millions of dollars spent trying to develop molecular standards in an attempt to replace it. [14] Currently, the use of computerized analysis of histological characteristics of prostate cancer is not new [15]. However, with advances in integral imaging capability and advanced computing technologies, an increasing number of artificial intelligence (AI) studies in prostate cancer pathology are being undertaken [16-17]. A group of researchers reported their findings using an artificial intelligence (AI) software system, called Paige prostate, to detect prostate cancer. In this study, which involved 1876 full slide images of prostate biopsy samples, the use of AI software demonstrated high sensitivity (97.7%) and high specificity (99.3%) in detecting PC.[18] In particular, Paige prostate can be beneficial for pathologists who are not specialized in prostate cancer, because they have achieved results as accurate, when using Paige, as specialists who have not used the system. Based on the results of this study, in September 2021, the United States Food and drug Administration (FDA) approved the Paige prostate system to assist pathologists in making a better and more accurate histological diagnosis of PC [18]. However, there are questions about the current capabilities of AI systems to distinguish treated prostate cancer, which can exhibit numerous other histological changes following multiple treatment lines, benign conditions that mimic prostate cancer, atypical lesions from prostate biopsies. I believe that the research in diagnosing PC has evolved grately, but in my opinion, Paige or any other AI system cannot replace the diagnosis made by pathologists today, but can improve the efficiency and accuracy of the pathological diagnosis of PC.

II. Material and method

Scope of this study is to evaluate both the upstaging and downstaging of the Gleason score and to compare the result of the conventional needle biopsy (NB) vs. radical prostatectomy (RP) scores.

Given the increased frequency of PC and the multitude of therapeutic variants, at the International Society of Urological Pathology (ISUP) consensus in 2019, the classification for the classification of PC cases in risk groups was established, thus emphasizing the importance of improving the correlation of Gleason score, both total and individual patterns, in the case of NB.

The first objective of the paper is to study the under staging and over staging of the Gleason score on conventional NB and to compare it with the Gleason score resulting from RP, both for the total Gleason score and then for each individual pattern.

The second objective of the paper is to study the under staging and over staging of the Gleason score on the BxChipTM biopsy and to compare it with the Gleason score resulting from RP, both for the total Gleason score and for each individual pattern.

The study carried out in this doctoral thesis is a retrospective study and was conducted within the urology department of the Clinical Hospital "Prof. Dr. Theodor Burghele" – Bucharest. 341 prostate cancer patients were admitted to the clinic between 2010 and 2016, of which 51 (2010-2011) had conventional NB and 291 (2012-2016) had NB with BxChipTM. Contact anesthetic (lidocaine gel 2g) was used and biopsy was done with an 18G needle, performed under transrectal ultrasound guidance (TRUS). In 2010, Sextant biopsy was used to a large extent, which was then shown to correlate with the higher rate of negative NB and a higher Gleason rate for positive NB, but since 2012, the most used was the 12-fragment biopsy, this results in a better correlation between the NB Gleason score and the RP Gleason score. The collected fragments were stored in groups (2 tubes of 3 fragments each in the case of sextant NB and 6 fragments respectively in each tube in the case of 12 fragment NB) for patients analyzed in the years 2010-2011, and for patients after 2012, the fragments were immediately stored in Themis Biopsy CHIP (Fig.1), which allowed for bulk analysis of all the fragments obtained from NB, respecting the topography of the harvest.

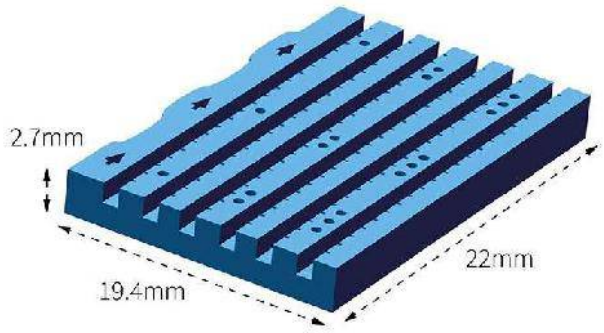

Figura1. Bx Chip ™ cu diametrul de 22 mm X 19,4 mm X 2,7 mm

Themis Biopsy Chip is a sectional and patented biomimetic matrix with 1 mm wide grooves that can accommodate 6 prostate biopsy cores on a single chip. The chip diameter is 22 mm X 19.4 mm X 2.7 mm. The properties of the chip allow it to be processed, incorporated and sectioned similarly to human tissue. The breakers between each trench allowed the precise location of the biopsy to be identified. BxChipTM received patent no. US 9.851, 349 B2. Due to the fact that BxChipTM has shown its effectiveness, it is currently used in many clinics both in Romania and in Europe. The pathology examination of the fragments collected by NB and the pieces resulting from RP was performed in the pathology laboratory of the "Prof. Dr. Theodor Burghele" Clinical Hospital, and all the histopathological results were interpreted by 3 pathologists. Excluded patients were those whose Gleason score could not be documented before surgery, as well as those whose pathology examination was performed in another clinic. Patients who already started neoadjuvant therapy have also been excluded, reason being that it could interfere with the postoperative Gleason score. For each patient, preoperative staging was performed by analyzing the NB fragments and determining the Gleason score. The RP piece was postoperatively fixed in 10% formaldehyde, reduced to paraffin, marked with China ink, microtome sectioned to 2-3 microns standard colored with Hematoxylin Eozin and Van Gieson.

Both diagnosis and management of PC are a significant burden on health systems worldwide due to the increased incidence and prevalence of the disease.

The active surveillance and diagnostic strategy, involve the systematic collection of 12 fragment NB, and in addition, we find an increased prevalence of targeted biopsies, guided by mpMRI.

The accuracy of Gleason staging and the correct quantification of the tumour percentage in each NB, are critical in patient management and imperative requirements in the NB report.

The intact and unfragmented assessment of the NB product is of great importance, but unfortunately fragmentation is common in standard NB processing due to the <1 mm diameter of the harvested cores. They are subject to physical stress factors when transferring from the biopsy needle to the formalin container and when transferred for fixation to the paraffin block.

An accentuation of these factors is found in biopsies with high tumor percentage or increased tumor grade, due to the decrease in the thickness of the connective tissue of stromal support.

As long as nonlinear fragmentation and alignment errors of the resulting NB are eliminated by fixation in the matrix, the time required for tissue orientation and tumor quantification is shortened.

The fragmentation of NB products can compromise diagnostic accuracy, through errors in establishing tumor grade and incorrect quantification of tumor percentage, which can lead to incorrect treatment of patients by misclassification into risk groups.

In the second study, we can observe in the ISUP classification, a degree of concordance between the support matrix NB and RP of 46.7%, increasing from 23.5% in the case of classical NB.

The improvement of the consistency with over 23% between the two groups of patients is also found due to the increase in the number of fragments collected by NB, as well as the improvement of the learning curve, both of the clinicians and the pathologists.

The same study, while showing a total Gleason score downgrading of 12.4% (36 patients) for matrix-supported biopsies, is well below that found in classical PBP of 35.3% (18 patients).

Precisely for these reasons, I believe that it is necessary to further study these differences, in order to be able to quantify as accurately as possible the role that the support matrix has in increasing the diagnostic concordance.

The Gleason biopsy scores were compared and the logistic ordinal regression analyses showed a greater consistency in the BxChipTM group versus the conventional NB group, the difference being statistically significant in all categories (Gleason, Major pattern, Minor

pattern, ISUP), thus, we can conclude that the use of BxChipTMM in medical practice, is a real innovation in the correct classification of PC.

According to the EAU guidelines, patients with diagnosed intermediate-risk PC and grade 3 ISUP should be excluded from active surveillance protocols, and will be exposed to treatment variants consisting of RP, nerve sparing RP in patients with low risk of extracapsular development, brachytherapy or external radiotherapy combined with short-term androgenic deprivation therapy.

The paper aims to highlight the differences in Gleason score, between NB and RP results. Emphasis will be placed on the study of methods leading to an increase in concordance between pre- and post-operative Gleason scores. The paper focuses both on the study of age groups and on comparisons of each architectural pattern.

According to NCCN guidelines, patients with intermediate-risk PC are subdivided into groups, in favourable intermediate risk and unfavourable intermediate risk, based on the ISUP score and the number of positive cores at NB, which emphasizes the importance of increasing the correlation between Gleason scores.

III. Results

STUDY I

The average age of patients in the study group was 64.21 years, with a standard deviation of 5.402, with age limits between 39 and 78 years and with the highest number of patients in the 56-70-year segment

Relative to PSA parameters, the most commonly reported values were between 7 - 8 ng/dL and > 10ng/dL, with an average PSA on the studied group of 9.7 ng/dL, with standard deviation of 7.82 reflecting an increased variability range of PSA values, between 3.01 ng/dl – 52.16 ng/dl.

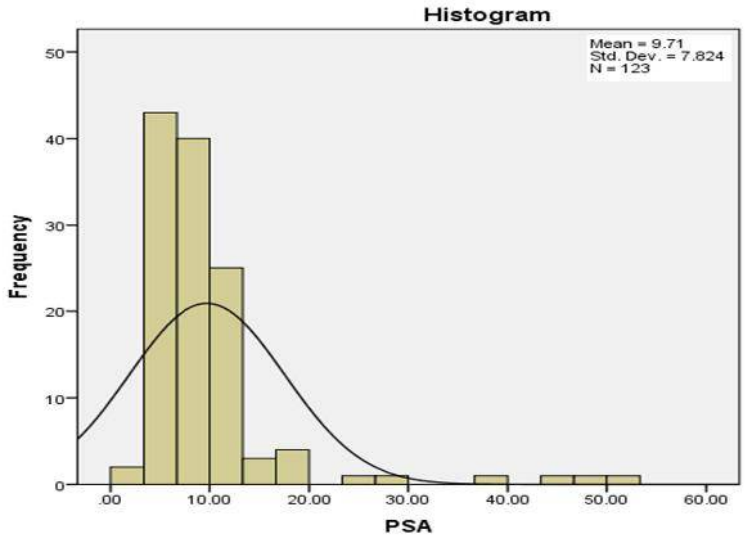

Figure 1. PSA value in patients in the study group

On NB specimens, Gleason 6 score was found in 33 patients (26.8%), Gleason 7 in 73 patients (59.3%), Gleason 8 in 16 patients (16%) and Gleason 9 in one patient (0.8%).

On RP specimens, Gleason 6 score was found in 17 patients (13.8%), Gleason 7 in 91 patients (74%), Gleason 8 in 6 patients (4.8%) and Gleason 9 in 9 patients (7.3%).

There were no Gleason 5 and 10 values at NB and in the case of RP.

		GS_NB		GS_RP	
Gleason Score		Frequency	Percent	Frequency	Percent
Valid	6.0	33	26.8	17	13.8
	7.0	73	59.3	91	74.0
	8.0	16	13.0	6	4.9
	9.0	1	.8	9	7.3
	Total	123	100.0	123	100.0

Table 1. The frequency of the Gleason score in patients with NB and RP.

The highest concordance between NB and RP was found in the Gleason 7 score, where the results were similar in 59 patients (80.8%).

There were 33 patients with Gleason score 6 per NB, of which 21 postoperatively showed an increase, with a final score of Gleason 7 (67.7%).

The tendency for upgrading occurs with high Gleason values (Gleason 8 and 9), where of 16 patients diagnosed with PBP with Gleason 8, 11 (68.8%) had Gleason final score ≤7, a patient who had a NB grade 9 on the RP specimen was 7 (100%).

The above data show the clear trend of NB downgrading for low Gleason values, the trend gradually disappears as this parameter increases, reaching the peak of concordance (80.8%) in the case of Gleason 7, followed by an upgrading trend in the case of Gleason values of 8 and 9.

STUDY II.

The patients ages ranged from 47 to 80 years with an average of≅ 65 years (64.74±5.86). The BxChip group has an average age (m65≅; sd=5.75) higher than for the Classic group (m63.5≅; sd=6.42), but following the application of the comparison test t for independent samples, the age distribution for the two lots is assumed to> be equal (t(338)=1.68 and p. We take into account that the two samples are part of a population aged between 45 and 80 years and with an average age that can vary between 64 and 65.5 years (C.I. 95% 64 ÷ 65.5)

Group	*N*	min	max	*M*	*Sd*	*sk*	*k*	*t* (df)	*p*
BxChip	290	47	80	64.97	5.75	-.21	-.10	1.68 (338)	NA
Classic	50	50	75	63.46	6.42	-.20	-.57		
Total	340	47	80	64.74	5.86	-.23	-.17	C.I. 95% (64.18 – 65.42)	

Note: N – number of subjects, m – arithmetic mean, sd – standard deviation, sk – asymmetry coefficient, k – vault coefficient, t – test value t for independent samples, df – degree of freedom, p – level of statistical significance, NA – p. >

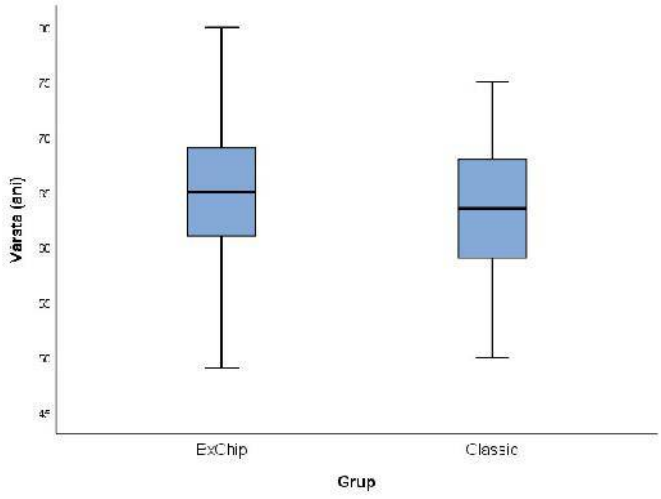

Figure 2. Comparative graph of age distribution by group

Lot	*N*	Min	max	*M*	*sd*	*sk*	*k*	*Extreme*
BxChip	288	2.60	95.00	12.01	11.73	4.19	22.46	≥22
Classic	50	2.50	48.00	9.30	7.78	3.65	15.25	≥19
Total	338	2.50	95.00	11.61	11.27	4.25	23.42	

Note: N – number of subjects, m – arithmetic mean, sd – standard deviation, sk – asymmetry coefficient, k – vault coefficient, t – test value t for independent samples, df – degree of freedom, p – level of statistical significance, NA – p. >

Table 3. Descriptive indicators of total PSA distribution

An extreme upper limit value of the range of variation in the BxChip group is observed, and extreme values starting at a value of approximately PSA=20 for both groups, which is why only cases with values between 2.50 and 20 will be used in subsequent analyzes.

Lot	*N*	min	max	*m*	*Sd*	*sk*	*k*	*t* (df)	*p*
BxChip	255	2.60	19.90	8.81	3.66	.90	.40	1.61 (301)	NA
Classic	48	2.50	19.00	7.89	3.45	1.10	1.19		
Total	303	2.50	19.90	8.67	3.63	.92	.46	C.I. 95% (8.26 – 9.08)	

Note: N – number of subjects, m – arithmetic mean, sd – standard deviation, sk – asymmetry coefficient, k – vault coefficient, t – test value t for independent samples, df – degree of freedom, p – level of statistical significance, NA – p. >

Table 4. Descriptive indicators of total PSA distribution excluding extreme values

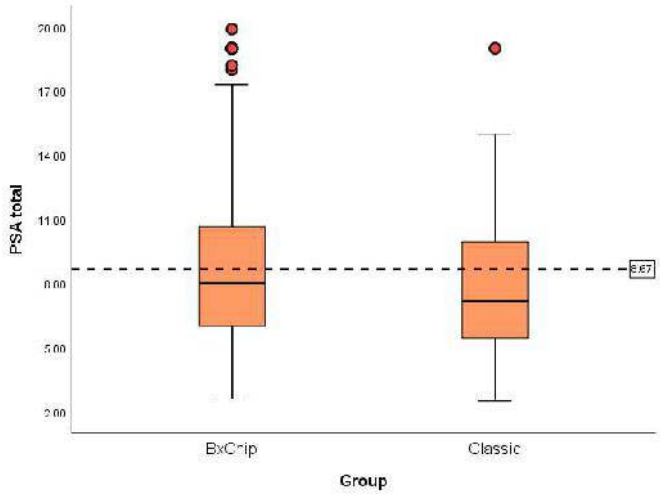

Figure 3. Comparative graph of the distribution of total PSA values by group

Group	Number of biopsies	10	11 – 12	13 – 15	> 15	Total
BxChip	N	9	249	14	19	291
	%	3.1	85.6	4.8%	6.5%	
Classic	N	27	22	2	0	51
	%	52.8	43.2	4.0	0.0	

Table 5. Analysis of the number of biopsies and the number of blocks analyzed

The two groups were compared based on the Gleason score obtained from the initial NB.

The comparative analysis was performed using the ordinal logistic regression in order to identify a trend difference in the level of severity diagnosed after the biopsy.

The analysis was performed only for those Gleason score types for which the Chi-square frequency distribution comparison test showed statistically significant differentiation.

Table 6. Description table of the distributions of the Gleason, Major pattern, Minor pattern, ISUP categories measured from NB on the two batches.

		BxChip		Classic		Total			
	Category	N	%	N	%	N	%	χ^2	*P*
Gleason	5	0	**0.0**	2	**3.9**	2	**0.6**	11.26	.010
	6	32	**11.0**	14	**27.5**	46	**13.5**		
	7	191	**65.6**	27	**52.9**	218	**63.7**		
	8	61	21.0	7	13.7	68	19.9		
	9	7	2.4	1	2.0	8	2.3		
Major	2	0	**0.0**	1	**2.0**	1	**0.3**	.47	NA
Pattern	3	162	**55.7**	30	**58.8**	192	**56.1**		
	4	128	44.0	20	39.2	148	43.3		
	5	1	0.3	0	0.0	1	0.3		
Minor	2	0	**0.0**	1	**2.0**	1	**0.3**	9.18	.010
Pattern	3	93	**32.0**	27	**52.9**	120	**35.1**		
	4	192	**66.0**	22	43.1	214	**62.6**		
	5	6	2.1	1	2.0	7	2.0		
ISUP	1	32	**11.0**	16	**31.4**	48	**14.0**	16.54	.002
	2	130	**44.7**	15	**29.4**	145	**42.4**		
	3	61	21.0	12	23.5	73	21.3		
	4	61	21.0	7	13.7	68	19.9		
	5	7	2.3	1	2.0	8	2.3		

Note:χ^2– value of the Chi-square test, p – the level of significance of the test value.

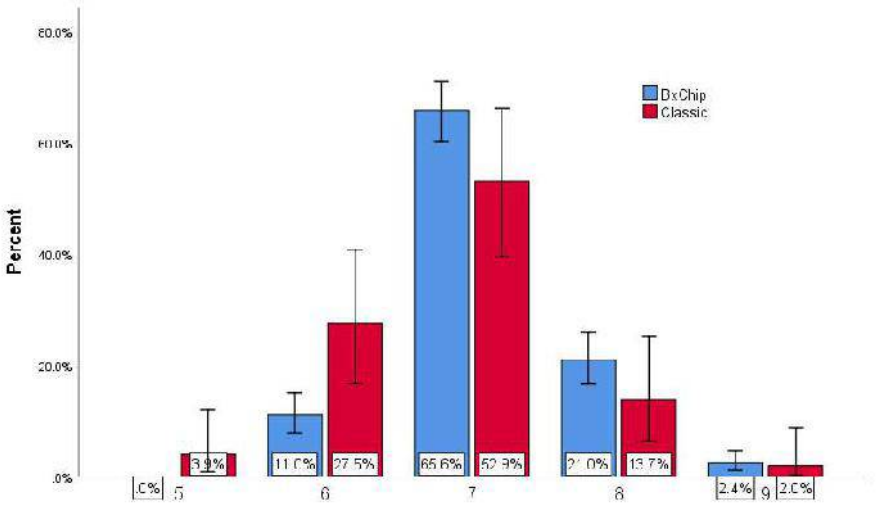

Figure 4. Gleason score median on the batch with BxChip vs Classic

Table 7. Description table of the distributions of Gleason, Major pattern, Minor pattern, ISUP categories measured by RP on the two batches.

		BxChip		Classic		Total			
	Category	N	%	N	%	N	%	χ^2	*p*
Gleason	6	7	2.4	3	5.9	10	2.9	4.76	NA
	7	259	**89.0**	40	**78.4**	299	**87.4**		
	8	11	3.8	3	5.9	14	4.1		
	9	14	4.8	5	9.8	19	5.6		
Major	3	141	**48.5**	29	**56.9**	170	**49.7**	1.36	NA
Pattern	4	149	**51.2**	22	**43.1**	171	**50.0**		
	5	1	0.3	0	0.0	1	0.3		
Minor	3	132	**45.4**	17	**33.3**	149	**43.6**	4.17	NA
Pattern	4	146	**50.2**	29	**56.9**	175	**51.2**		
	5	13	4.4	5	9.8	18	5.2		
ISUP	1	9	3.1	3	5.9	12	3.5	5.73	NA
	2	134	**46.0**	26	**51.0**	160	**46.8**		
	3	122	**41.9**	14	**27.5**	136	**39.8**		
	4	12	4.1	3	5.9	15	4.4		
	5	14	4.8	5	9.8	19	5.6		

Note:χ^2– value of the Chi-square test, p – the level of significance of the test value.

Comparative analyzes of the distribution of RP-NB degree differences for Gleason, Major pattern, Minor pattern, and ISUP by lot.

The difference between the degree obtained from the RP and the degree obtained from the NB was coded as follows:

- Up (1, 2, >2) – NB Gleason grade > RP Gleason grade, upgrading
- Same – grade identity
- Down (1, 2, >2) – NB Gleason grade < RP Gleason grade, downgrading

Table 8. Descriptive table of the distributions of the Gleason differences on the two lots.

		Difference Gleason total							
		up 2	up 1	Same	down 1	down 2	Total	χ^2	*p*
BxChip	Count	5	45	201	36	4	291	27.64	.001
	%	1.7%	**15.5%**	**69.1%**	**12.4%**	1.4%	100.0%		
Classic	Count	0	7	22	18	4	51		
	%	0.0%	13.7%	**43.1%**	**35.3%**	**7.8%**	100.0%		
Total	Count	5	52	223	54	8	342		
	%	1.5%	**15.2%**	**65.2%**	**15.8%**	2.3%	100.0%		

- Note:χ^2– value of the Chi-square test, p – the level of significance of the test value.

Table 9. The results of the analysis of the ordinal logistic regressions having as a factor the type of NB applied

				Hypothesis Test				95% Wald Confidence Interval for Exp(B)	
	Parameter	B	Std. Err	Wald Chi-Square	df	Sig.	Exp(B)	Lower	Upper
ISUP	[lot=2]	.41	.10	**16.60**	**1**	**.001**	**1.51**	1.24	1.84
	[lot=1]	0a	.	.	.	.	1	.	.

Following the analysis of the ordinary logistic regression, a 1.5 (C.I. 95% 1.24 ÷ 1.84) times higher chance of obtaining downgrading in the classical NB lot than in the NB batch performed with BxChip. The trend is statistically significant for a Wald test valueχ 2=16.60, df=1, and p.<001.

Table 10. Descriptive table of the distributions of the ISUP differences on the two batches.

		Difference ISUP									
		up >2	up 2	up 1	same	down 1	down 2	down >2	Total	χ^2	*P*
BxChip	N	3	16	49	136	77	10	0	291	20.31	.002
	%	1.0%	**5.5%**	**16.8%**	**46.7%**	**26.5%**	**3.4%**	0.0%	100.0%		
Classic	N	0	1	12	12	20	5	1	51		
	%	0.0%	2.0%	**23.5%**	**23.5%**	**39.2%**	**9.8%**	**2.0%**	100.0%		
Total	N	3	17	61	148	97	15	1	342		
	%	0.9%	5.0%	17.8%	43.3%	28.4%	4.4%	0.3%	100.0%		

Note:χ ^{2}– value of the Chi-square test, p – the level of significance of the test value.

For ISUP scores, BxChip patients identified a major trend of equality between NB and RP scores (46.7%) and a slight accentuation of downgrading (26.5%), while the major trend in classical NB is downgrading (down1 and 2) (≅49%), and the equality of scores was observed in only 23.5% of cases. The trend difference is statistically significant for a test valueχ 2=20.31 df= p.01<.

Concordance analysis of Gleason NB – RP scores

The concordance analysis was carried out using the web application VassarStat (Kappa (vassarstats.net), the value of the Kappa coefficient was determined using three weighting variants: without weighting (considering only absolute concordances), with linear weighting (considering also the relative concordances according to the distance from the position of the absolute concordance), and square weighting through the squares of the linear weights)

Table 11. Gleason NB-RP score association table for the BxChip group

Frequency of concordance	The proportion of concordances % C.I. 95%

Gleason score	Maximum possible	Expected	Observed	Maximum possible	Expected	Observed	Limit lower	Limit upper
6	7	.77	5	21.9	2.0	14.7	5.5	31.8
7	191	170	184	73.8	60.7	69.2	63.2	74.6
8	11	2.31	8	18.0	3.3	12.5	5.9	23.7
9	7	.34	4	50	1.6	23.5	7.8	50.2
Total	216	173.42	201	74.2	59.6	69.1	63.4	74.3

The confidence interval (C.I. 95%) for proportions was calculated with Wilson continuity correction.

Kappa coefficient value

Method of calculation	Kappa	Standard error	C.I. 95%	
			Lower limit	Upper limit
Absolute	**.235**	.067	.103	.366
Linear weighted	**.288**	.054	.182	.394
Square weighted	**.369**	.041	.290	.449

Table 12. Gleason biopsy-prostatectomy score association table for the Classic group

Score Gleason	Frequency of concordance			The proportion of concordances %			C.I. 95%	
	Maximum possible	Expected frequencies	Observed	Maximum possible	Expected	Observed	Lim Lo	Lim Up
6	3	.86	1	21.4	5.3	6.3	0.3	32.3
7	27	20.9	20	71.1	47.5	44.4	30.0	59.9
8	3	.43	0	42.9	4.5	.0	0	34.5
9	1	.10	1	20	1.7	20.0	1.1	70.1
Composite	34	23.33	22	69.4	45.6	44.9	30.9	59.7

The confidence interval (C.I. 95%) for proportions was calculated with Wilson continuity correction.

Since the weight of observed concordances (44.9%) is lower than the weight of randomly obtained concordances (45.6%) it was not possible to calculate the concordance coefficient.

Table 13. ISUP NB-RP grade association table for the BxChip group

Gleason score	Frequency of concordance			The proportion of concordances %			C.I. 95%	
	Maximum possible	Expected	Observed	Maximum possible	Expected	Observed	Lim. lower	Lim. upper
1	9	1.0	5	28.1	2.5	13.9	5.2	30.3
2	130	59.9	81	97.0	29.3	44.3	37.0	51.8
3	61	25.6	38	50.0	16.3	26.2	19.4	34.3
4	12	2.5	8	19.7	3.6	12.3	5.8	22.4
5	7	.3	4	50	1.6	23.5	8.8	50.2
Total	219	89.3	136	75.3	30.7	46.7	40.9	52.6

The confidence interval (C.I. 95%) for proportions was calculated with Wilson continuity correction.

Kappa coefficient value

Method of calculation	Kappa	Standard error	C.I. 95%	
			Lower limit	Upper limit
Absolute	**.232**	.042	.150	.314
Linear weighted	**.348**	.040	.271	.426
Square weighted	**.479**	.078	.327	.632

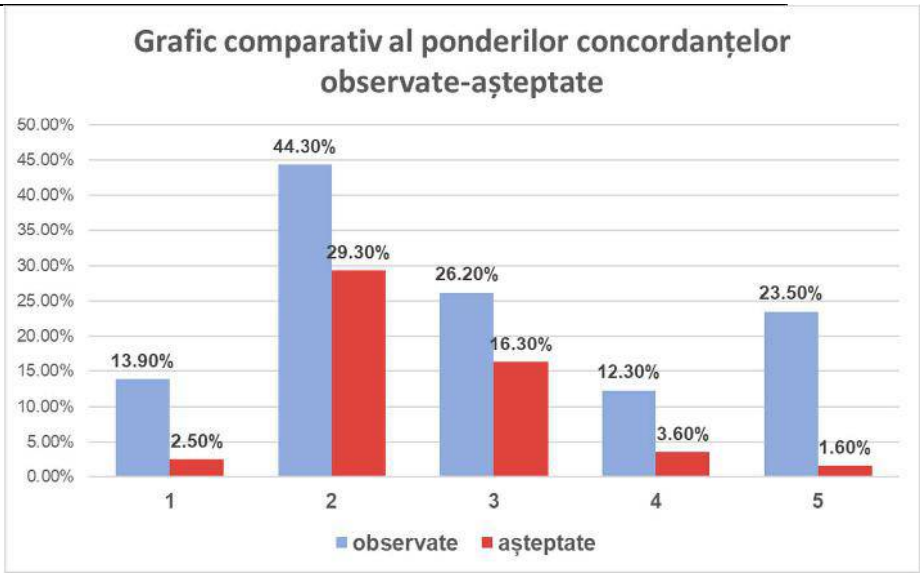

Figure 5. Comparative graph of observed-expected concordance weights

Table 14. ISUP NB-RP grade association table for the Classic group

Score Gleason	Frequency of concordance			The proportion of concordances %			C.I. 95%	
	Maximum possible	Expected frequencies	Observed	Maximum possible	Expected	Observed	Lim Lo	Lim Up
1	3	.9	1	18.8	5.2	5.6	.3	29.4
2	15	7.7	8	57.7	22.9	24.2	11.7	42.6
3	12	3.3	2	85.7	14.5	8.3	1.5	28.5
4	3	.4	0	42.9	4.3	0	0	34.5
5	1	.1	1	20	1.7	20	1.1	70.1
Composite	34	12.4	12	66.7	24.3	23.5	13.3	37.8

The confidence interval (C.I. 95%) for proportions was calculated with Wilson continuity correction.

Since the weight of observed concordances (23.5%) is lower than the weight of randomly obtained concordances (24.3%) it was not possible to calculate the concordance coefficient.

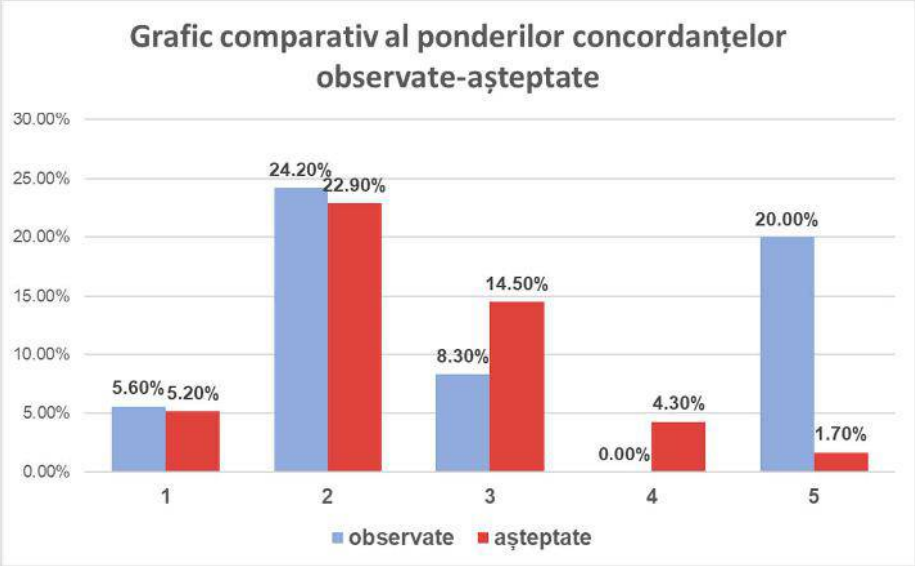

Figure 6. Comparative graph of observed-expected concordance weights

IV. Discussions

Both diagnosis and PC management constitute a significant burden on health systems worldwide due to the increased incidence and prevalence of the disease.[156]

Active surveillance and diagnostic strategy involves systematic NB collection of 12 cores, and additionally, we find an increased prevalence of targeted NB guided by multiparametric MRI.[158]

The accuracy of Gleason grading and the correct quantification of the tumor percentage in each NB fragment are critical in patient management and imperative requirements in the NB report.[159]

Intact and unfragmented assessment of the NB specimen is of great importance, but unfortunately fragmentation is frequently encountered in standard NB processing, due to the diameters of < 1mm of the cores harvested; they are subject to physical stress factors when transferring from the biopsy needle to the formalin container and when transferring to the paraffin block.[160-162]

An emphasis on these factors is found in NB cores with a high tumor percentage or increased tumor grade, due to a decrease in the thickness of the stromal support connective tissue.[163]

As long as nonlinear fragmentation and alignment errors of the resulting NB specimen are eliminated by fixation in the matrix, the time required for tissue orientation and tumor quantification is shortened.[164]

Fragmentation of the NB cores may compromise diagnostic accuracy through errors in establishing tumor grade and incorrect tumor percentage quantification, which may lead to incorrect treatment of patients by misclassifying them into risk groups.[165]

The study presented at the Annual Conference of the American Urology Association (AUA) in 2016 by K.J. Wojno and collaborators, on 267 biopsies, a tissue surface to be examined on the slide is an average of 5.5 mm2 and an average length of fragments of 14.1mm, in the case of fragments used in the matrix, increasing from 4.8 mm2 and 10.7mm in length respectively for fragments harvested and processed without matrix; this results in an increase in the neoplasic detection rate from 49.5% to 58.8% in the case of fragments used in the matrix.

From the start, we can see remarkable scientific progress between the treatment options of PC currently available, compared to those in 2010-2011. Research is constantly developing in an attempt to find new, targeted therapies.

The patient is and will remain the main decision-making factor, the treatment must be individualized and thus respond to the patient's expectations; This is why research in PC focuses on interpreting and discovering the mechanisms involved in carcinogenesis, whether molecular or genetic. Expanding our understanding of these mechanisms, which highlights different types and molecular subtypes of PC, must translate into the development of new targeted therapies that take into account the loco-regional extension and morpho-functional characteristics.

In the second study, we can see that in the case of ISUP score, a degree of concordance is found between the support matrix NB and the RP specimen, of 46.7% increasing from 23.5% in the case of the classic NB. [166]

The improvement of the concordance of more than 23% between the two groups of patients is also found due to the increase in the number of fragments collected by NB, as well as the improvement of the learning curve of both the clinician and the pathologist. [167]

The same study, while showing a total Gleason downgrading of 12.4% (36 patients) for matrix-supported NB, is well below that found in classical NB of 35.3% (18 patients). [168]

It's precisely for these reasons that I believe it's necessary to further study these differences in order to be able to quantify as accurately as possible the role of the support matrix in increasing the diagnostic concordance.[169-170]

I. Conclusions

The significant increase in the Gleason grade on the RP specimen occurs especially in patients whose preoperative value was equal to 6, results that correlate with data communicated in the literature [2]. Gleason downgrading on the cores obtained by NB is important because it can radically influence the therapeutic decision, including the patient in the surveillance group and in this way the optimal surgical therapeutic moment can be exceeded. Overtreatment involves the inclusion of patients in the group of those who will benefit from RP, which has favorable prognostic implications, but with the risk of the presence of potentially disabling perioperative complications for the patient (urinary incontinence and/or erectile dysfunction).

In principle, a prostate tissue sample prelevated by NB cannot be representative of the whole gland and therefore scores will be different from that on the RP specimen. Based on our study, before initiating a "watchful waiting" protocol, a saturation puncture would be recommended to cover a larger area of prostate tissue.

Obviously, NB is downgrading, so prostate tumors limited to the organ, diagnosed by biopsy, in the case of low Gleason grade (=6), have an increased risk of being at an upper grade on the RP specimen.

Gleason grades of the NB were compared, and logistical ordinal regression analyzes showed greater consistency in the BxChipTM group over the conventional NB group, the difference being statistically significant in all categories (Gleason, Major pattern, Minor pattern, ISUP), thus we can conclude that the use in medical practice of the BxChipTM support matrix in medical practice, is a real innovation in staging and then the correct classification into evolutionary risk groups of prostate cancer.