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Significance of AMACR Expression as Additive Factor to Bone Scan, Gleason's Score and PSA in predication of therapy response in advanced Prostatic Carcinoma

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ABSTRACT

Prostate cancer is one of the popular male genital malignancies worldwide. Several studies have interested on the impact of AMACR expression as indicative and prognostic marker for prostate cancer. Many researches focused on Gleason's score, PSA and bone scintigraphy in diagnosis and prognosis of prostate cancer. Our aim is to explore the immune histo-chemical expression of AMACR as additional prognostic and predictive marker in prostate cancer. **Material and Methods**: Immune histo-chemical staining of AMACR on tissue core biopsies of 49 patients with advanced prostatic cancer and evaluated its expression in relation to Gleason's score, initial PSA level, nadir PSA level and bone scan as well as biochemical failure to androgen deprivation therapy. **Results:** we found statistically significant relation between high AMACR expression and high Gleason's score. Patients with high AMACR expression have a tendency to present with higher level of PSA and positive bone deposits. A tendency was identified for patients with AMACR score 7 to have higher nadir PSA. Biochemical failure was depicted in 71.4% of patients with AMACR Score 7, while patients with AMACR score less than 7 didn't had biochemical failure. On follow up bone scan, ten patients get progression and the AMACR score in these patients was 7.

Conclusion: Our initial results focused on the role of AMACR immune expression as a poor prognostic indicator of prostate cancer, being linked with high Gleason's score, increased incidence of positive bone metastases and initial high PSA level. In addition, the results highlighted the potential role of AMACR expression as predictive marker of hormonal treatment.

Key words: AMACR, Advanced prostatic cancer, Gleason's score, Prognosis.

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INTRODUCTION:

Prostate cancer is one of the popular malignancies in men worldwide, about 220,800 new cases and 27,540 deaths was recorded in 2015 [(1)]. In Egypt, prostatic cancer is the more common male genital cancer (60.7%) at the NCI, Cairo University in the last decade [(2)]. Alpha-Methylacyl- CoA- Racemase (AMACR; P504S) has an essential role in the ß-oxidation of branched-chain fatty acids and fatty acid derivatives as it catalyzes the transformation of several 2R)-methyl-branched-chain fatty acyl-CoAs to their (S)-stereoisomers [(3)]. The gene for AMACR is located on chromosome 5p13 and encodes a 382 amino acid protein [(4)]. The bone is first and main site of metastases in prostatic carcinoma (about 80%), it is considered one of the essential prognostic factors [(5, 6)].

Several guidelines consider 99mTc bone scan as the main investigative techniques to identify and monitor bone metastases [(7,8)]. Bone scan is done primarily if the PSA is high, the Gleason score is >7 or in symptomatic patients. However, bone scan should be acquired in patients with poorly differentiated tumors and locally advanced disease, regardless of the serum PSA value [(8)]. The bones scan sensitivity range between 62 and 89% and then it considered acceptable. Androgen ablation by means of castration and/or administration of small chemical inhibitors (e.g., luteinizing hormone-releasing hormone agonists and AR antagonists) is the usual management for advanced prostatic cancer [(9)]. Several studies have interested on the importance of AMACR expression as an investigative marker for

Prostatic carcinoma, especially in context of differential diagnosis with benign prostate mimics [10-15].

Lin and colleagues reported the relation of serum level of AMACR can clearly differentiates healthy males from men with high-grade prostatic intraepithelial neoplasia (HGPIN) and highlighted its role in the pathogenesis of prostate cancer [16]. Moreover, ***Gumulec and colleagues*** found that the AMACR level is clearly higher in patients with Gleason score 9. The present study aimed to evaluate the role of immune histo-chemical expression of AMACR in prostatic cancer and its relation as regards well established prognostic indicators especially osseous deposits and response to treatment [17].

MATERILAS AND METHODS:

This retrospective study was carried on 49 cases of locally advanced and metastatic prostatic carcinoma during the period from 2011-2016. Data of cases was collected from medical archives at different departments in NCI, Zagazig University Hospitals and Fayoum University Hospitals. Histopathological examination of the slides of all patients was reviewed at the Pathology Department, National Cancer Institute (NCI), Cairo University.

All patients underwent sub-capsular orcheidectomy and received anti-androgen (Casodex). Moreover, when progressed on hormonal treatment they received chemotherapy (Taxotare) if tolerated. Hematoxylin and eosin (H&E) stained slides were reviewed. Histopathological examination was done for revision and confirmation of diagnosis and categorization. Tumors were scored according to the criteria described at the 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma [14]. One positively charged unstained slide 5 micron thick, was prepared from paraffin blocks and stained with AMACR using ready to use monoclonal rabbit antibody against AMACR, clone 13H14 (Dako Denmark A/S). Immunostaining was performed using autostainer BenchMark IHC/ISH staining module (Ventana). Slides stained with AMACR were evaluated for intensity and attribution of carcinoma cells stained. Intensity score was rated 0 (non-circumferential staining), 1+ (focal apical granular staining), 2+ (diffuse weak cytoplasmic staining), or 3+ (strong, cytoplasmic and luminal staining).

Proportion was rated with respect to percentage of positively stained cells, as follows: 0 (<5%cells stained), 1+ (5% to 25% of cells stained), 2+ (26% to 50% of cell stained), 3+ (51% to 75% of cells stained), and 4+ (76% to 100% of cells stained). The intensity and proportion scores were added to give an overall score, with 7 being the highest possible. All scores>0 were considered AMACR positive [15]. Correlation with other clinic-radiological data, bone scintigraphy and laboratory data was done.

Statistical methods: Data was analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data was expressed as mean and standard deviation or median and range as appropriate. Qualitative data were displayed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc "Schefe test" was used for pair-wise comparison based on Kruskal-Wallis distribution. Spearman-rho method was used to test correlation between numerical variables. Survival analysis was used for time to events variables. It was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Kappa test was used to evaluate agreement between two prognostic scores. All tests were two-tailed. A p-value < 0.05 was considered significant.

RESULTS:

Clinico-pathologic data of all patients are listed in ***Table 1***. Median age of our cases is 69.2 ranging from 54 to 90 years. The majority of patients were of Gleason's score 7 (42.9%). Initial PSA level was available for 38 cases with a mean of 586.5 ng/ml. 26 patients (53.1%) had positive bone scan.

Table1: clinic-pathologic characteristics of 49 patients with prostate cancer.

Age	Median:69 [54-90years]
Bone metastasis by bone scan at initial presentation	
Yes	26(53.1%)
No	15(30.6%)
Unknown	8(16.3%)
Gleason's score:	
6	8 (16.3%)
7(3+4)	10(20.4%)
7(4+3)	11(22.4%)
8	12(24.5%)
9	6(12.3%)
10	2(4.1%)
AMACR Score:	
1-6	19(38.8%)
7	30(61.2%)
Mean initial PSA	586.5 ng/ml [0.1-7500]

High statistically significant relation was obtained when comparing Gleason's score with AMACR score, p= 0.004. 70.7 % of patients with Gleason's score equal or more than 7 were of AMACR score 7 ***(Figures 1, 2, 3)***. Most of patients with AMACR score 7 (72%) presented with bone metastasis, while 50% of patients with AMACR score 1-6 presented with bone metastasis (p=0.154), three patients presented with super scan pattern initially, most of bone metastases were sclerotic in nature.

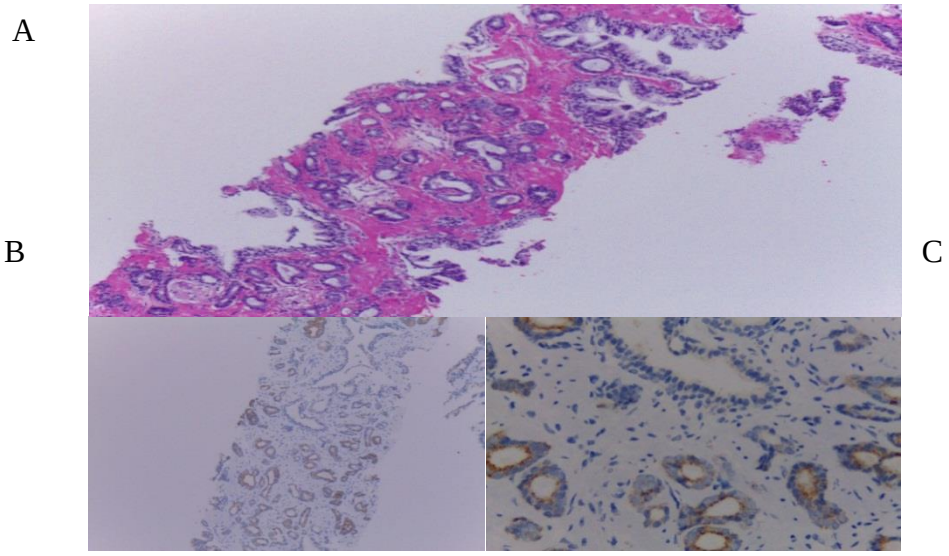

Figure (1): A: A case of prostatic adenocarcinoma, Gleason's score 3+3=6 [H&E x100]. B: The same case showing AMACR in apical pattern in 100% of the tumor cells, score 6 [x100]. C: High power of the same case. Note the restricted apical localization and the absence of AMACR expression in the benign prostatic glands [x400].

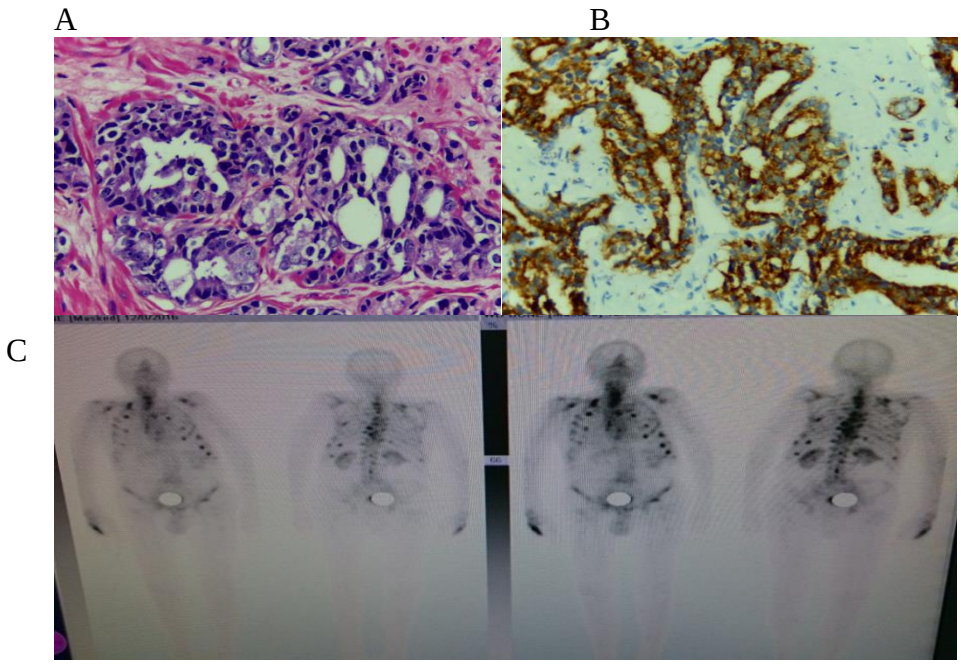

Figure (2): A: A case of prostatic adenocarcinoma Gleason's score 4+4=8. Note the cribriform pattern [H&E x 400). B: The same case showing AMACR expression in strongly intense both luminal and diffuse cytoplasmic localization, AMACR score 7 [x400]. C: the case showing multiple metastatic bone lesions at the initial bone scan.

A B

C

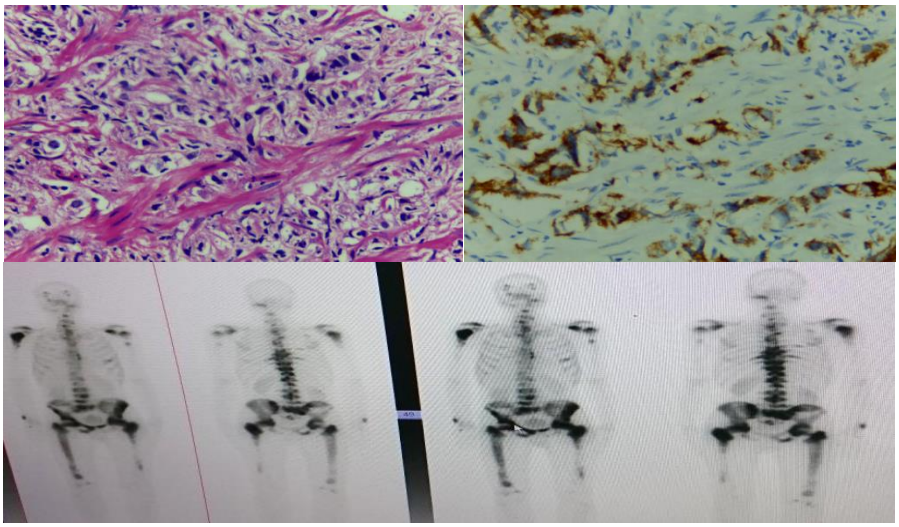

Figure (3): A: A case of prostatic adenocarcinoma Gleason's score 5+5=10. Note diffuse sheets of single cells [H&E x400]. B: the same case showing AMACR expression in diffuse intense cytoplasmic localization. AMACR score 7 [x400]. C: the case showing metastatic super scan pattern on bone scan.

There is significant difference between AMACAR score and Gleason's score more than 7.0 as compared to those less than 7.0 ($P < 0.004$). As regards initial PSA level, median level of PSA in patients with AMACR score 7 was 141.8 ng/ml, compared to 58.9 ng/ml in patients with AMACR score 1-6 (p=0.08). No relation was detected between AMACR score and bone metastases (p=0.15). ***(Table 2)***.

Table (2): Relation between AMACR score and Gleason's score, bone metastasis, initial PSA:

	AMACR score			
	Score 1-6	Score 7	Total	p value
Gleason's score:				
Score 6	7(87.5%)	12(29.3%)	19(38.8%)	0.004
Score 7,8,9,10	1(12.5%)	29(70.7%)	30(61.2%)	
Total	8	41	49	
Bone metastasis:				
Yes	8(50%)	18(72%)	26(63.4%)	0.154
No	8(50%)	7(28%)	15(36.6%)	
Total	16	25	41	
Initial PSA (ng/ml): Median [range]	58.9(0.1-1744)	141.8(9.9-7500)	104.4 [0.1-7500]	0.08

Nineteen patients were thoroughly and confidently followed as regards, nadir PSA level (lowest level the PSA drops after treatment), time to nadir and time to biochemical failure, that determined as elevation of PSA level more than 0.2 ng/mL (16). Three consecutive rises in PSA level were recorded to confirm biochemical failure. Mean follow up period is 16.28 months ranging from 2-48 months. A trend was noticed for patients with AMACR score 7 to have higher nadir PSA [1.4] in relation to patients with AMACR score 1-6 [0.02] with no significant difference (p= 0.09). ***(Table 3)***.

Table 3: Relation between AMACR score and nadir PSA and time to nadir PSA.

	Median [range]	**AMACR score**		p value
		1-6	7	
Nadir PSA (ng/ml)	0.1[0-1457]	0.02(.002-5.1)	1.4(0-1457)	0.09
Time to Nadir (months)	9[1-29]	13(1-20)	9(1-29)	0.9

Kaplan-Meier method was used to evaluate statistical relation between AMACR score and biochemical failure with PSA rise during follow up. As regards AMACR expression, none of patients with AMACR <7 experienced biochemical failure, while patients with AMACR score 7 experienced biochemical failure in 10/14 (71.4%) of cases. Median time to biochemical failure was 17 months [2-38] and median PSA level at time of failure was 62.3 (0.05-1920) ***(Figure 4)***.

As regards follow up bone scan, ten patients developed progression in bone metastases and they were of AMACR score 7, thirteen patients developed regression of the bone metastases with 12 patients were of AMACR score less than 7, While the remaining three patients were of stationary course on serial bone scan films. Seven patients died within 5 years and they had progressing bone metastases and AMACR score 7.

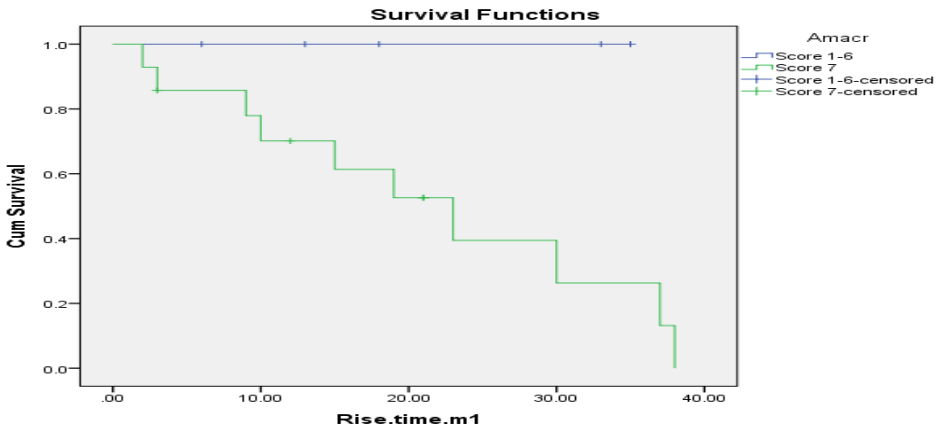

Figure 4: Relation between AMACR score and Time to PSA rise in months.

DISCUSSION:

AMACR is an enzyme that has important role in peroxisomal -oxidation of dietary branched-chain fatty acids and C27-bile acid intermediates [(18)]. The high rates of AMACR overexpression have been reported for prostate cancer, and in combination with basal cell markers, AMACR staining can be used with confident in differential diagnosis of controversial prostatic biopsies [(19 & 20)]. In our study, we targeted to test the role of the immune histo-chemical expression of AMACR as a potential prognostic and predictive marker of advanced prostatic cancer in relation to bone scan and other established prognostic factors. We reported high statistically significant relation between AMACR and Gleason's score as 70.7 % of patients of Gleason's score equal or more than 7 were of AMACR score 7 [p =0.004].

As regards initial PSA level, median level of PSA in AMACR score 7 was 141.8 ng/ml, compared to 58.9 ng/ml in cases with AMACR score 1-6 [p=0.08].

In addition the relation of immune histo-chemical expression of AMACR and effect of hormonal treatment we found that the median level of nadir PSA in AMACR score 7 was 1.4 ng/ml, compared to 0.02ng/ml in AMACR score 1-6 [p = 0.09]. The relation of AMACR to failure of hormonal treatment was evaluated as estimation of the rise of PSA level. None of patients with AMACR score < 7 experienced biochemical failure, while patients with AMACR score 7 showed biochemical failure in 10/14 (71.4%) of cases. Median time to biochemical failure was 17 months [2-38], while median PSA level at time of failure was 62.3 (p=0.06).

Along with our results, ***Gumulec and colleagues*** in their series AMACR in sera of 82 prostate cancer patients and concluded that AMACR level is clearly higher in the Gleason score 9 in serum of patients and in case of bone metastases [(13)]. On contrary, ***Rubin et al*** [(23)] explored the significance of AMACR as a biomarker for aggressive prostatic carcinoma in localized prostatic cancer. They showed that the AMACR expression is linked with prostate cancer progression in patients with clinically localized prostate cancer. The risk of prostate cancer death was 18-fold higher among those patients with low AMACR expression and high Gleason score (P = 0.006). Also, ***Barry et al,*** [(24)] explained that after treatment over a 20-year follow-up period, the development of metastatic and lethal prostate cancer was not independently linked with Low AMACR expression in primary tumor specimens, However they found that lower AMACR concentration was allied with greater prostate-specific antigen levels (p=0.003) and more advanced clinical stage (p=0.06) at diagnosis. These contradictory results could be lead to different scoring systems each study used to evaluate AMACR expression, as there is no agreement on cutoff point to separate cases as low and high expressers as most patients of prostatic carcinomas are expected to be positive. We suggest that our results relating AMACR expression to be a bad prognostic and predictive marker have strong rational. The early reports of AMACR expression in high-grade prostatic intraepithelial neoplasia [HGPIN] supports that it is implicated early in the course of the disease. Moderate to strongly positive staining has been reported for HGPIN in more than 64% of foci [(25)]. ***Jiang et al*** [(26)] also reported strongly positive staining in HGPIN. When HGPIN partially involved a gland, staining was confined to the HGPIN and did not outspread into the normal epithelial cells within the same gland. In addition, ***Luo et al*** [(27)] explained that invasive carcinoma and HGPIN together had more immune histo-chemical staining scores than normal prostate epithelium. However, the recorded score for carcinoma was significantly greater than that for HGPIN. Comparable results were also stated by ***Rubin et al.*** [(28)], which mentioned to the role of AMACR in progression of the prostatic cancer. Furthermore**,** ***Zha and colleagues*** [(8)] suggested that AMACR was functionally important for the progress of prostatic cancer. The enzymatic activity of AMACR was augmented 4-fold in prostatic carcinoma compared to the adjacent normal prostate tissue. Small interference RNA (siRNA) against

AMACR, but not the control-inverted siRNA, reduced the expression of AMACR and significantly reduced proliferation of the androgen-responsive prostatic carcinoma cell line LAPC-4. Moreover, concurrent inhibition of both the AMACR pathway by siRNA and androgen signaling by means of androgen withdrawal or anti-androgen suppressed the growth of LAPC-4 cells largely than either treatment alone. Taken together, these data suggested that AMACR is necessary for ideal development of prostatic carcinoma cells in vitro and that this enzyme has the potential to be a complementary target with androgen ablation in prostatic carcinoma treatment. Once AMACR protein is expressed, its level remains elevated as the prostatic carcinoma progresses to higher grades and stages and even metastasizes [(27)].

A detailed immune histo-chemical analysis utilizing both standard slides and tissue microarrays of samples from 168 primary prostate cancer cases, confirmed that both prostate carcinomas and the presumed precursor lesion (high-grade prostatic intraepithelial neoplasia), had significantly higher score than matched normal prostate epithelium; 88% of the prostate carcinomas had a staining score higher than the highest score observed for any sample of normal prostate epithelium.

Untreated metastases and wide spread bone metastases (n = 32 patients) as well as hormone refractory prostate cancers (n = 14 patients) were generally strongly positive for AMACR [(27)]. ***Takahara and colleagues*** [(27)] proposed that AMACR inhibition, which may induce characteristic transformation from a hormone-independent to dependent state, could be a hopeful novel planning for management of patients with hormone-refractory prostate cancer. Oral intake of AMACR inhibitors may be a treatment choice with potentially good compliance, Moreover, in vivo experiments involving local transfection of siRNA for AMACR into tumors resulted in a distinctive transformation from a hormone-independent to a hormone-dependent condition, leading to significant tumor regression. Finally, these experiments suggest the possibility of targeted gene therapy for patients with hormone-refractory prostate cancer as well as refractory bone deposits. A bone scan is important for prostate cancer staging, to identify possible bone deposits. However, a recent cross-sectional study exhibited lowly adherence to imaging guidelines, although applying a bone scan according to guidelines showed a well result. Inappropriate bone scan of patients at low risk of bone deposits and underuse

of bone scans in patients with high risk have also reported. [21]. In the light of EAU guidelines, a bone scintigraphy may not be recommended in asymptomatic patients with a well or moderately differentiated tumor if the serum PSA level is <20 ng/mL. As regarding the AUA guidelines, a bone scintigraphy is needless with local disease associated with serum PSA level <20 ng/mL and there is no clinical proof of bone metastases. The NCCN guidelines recommend bone scintigraphy for symptomatic patients and/or those with life expectancy >5 years when they have any of the following criteria: T1 disease with PSA >20 ng/mL or T2 disease with PSA >10 ng/mL, GS ≥8, or T3 or T4 or symptomatic disease. According to Briganti's CART, a bone scintigraphy should be indicated only for patients with a GS >7 or serum PSA level >10 ng/mL with a palpable tumor (cT2/T3). ***Ritenour and colleagues*** proposed that the PSA threshold should be adjusted according to the GS for recommending a bone scintigraphy in newly diagnosed patients with prostate cancer; bone scintigraphy was indicated for GS ≤7 and PSA >30 ng/mL and for GS ≥8 to 10 and PSA >10 ng/mL **(22)**.

In our research, most of the metastatic bone lesions were found in patients with AMACR score 7 or more and in Gleason's score more than 7 which is matched with most of guidelines. To our best of knowledge, our study is among the first that join the immune-histochemical expression of AMACR on tissue core biopsy with the hormonal treatment response in patients with advanced prostatic cancer and those with metastatic cancer prostate to bone.

CONCLUSIONS:

preliminary results suggest that AMACR immune-expression is a poor prognostic indicator of prostate cancer being accompanied with high Gleason's score and bone metastases at presentation as well as initial PSA level. In addition, our results highlighted the potential role of AMACR expression as predictive marker of hormonal therapy.

A small sample size may constitute the main limitation of our study, yet our sample was homogenous as regards stage at presentation and line of treatment as well as strict application of the definition of biochemical failure from all available clinical data.

Finally, we recommend further large-scale studies that relate immunohistochemical expression of AMACR to prognostic and predictive factors of prostatic carcinomas especially advanced cases, with special emphases on establishing a reproducible

Scoring system, to distinctly identify the value of the use of AMACR inhibitors as a valid effective line of treatment in cases of prostatic carcinoma.

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