AIM OF THE WORK

1- Assessment of AMACR expression in a wide range of prostatic carcinoma, high grade prostatic intraepithelial neoplasia (PIN) and benign prostate samples.
2- Assessment of p63 expression in a wide range of prostatic carcinoma, high grade prostatic intraepithelial neoplasia (PIN) and benign prostate samples.
3- Evaluate the utility of co-expression of AMACR and p63 in prostatic cancer , high grade prostatic intraepithelial neoplasia (PIN) and atypical prostatic lesions.

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AMACR	Alpha Methyacyl Co-A Racemase
ASL	Atypical small acinar lesion
CT	Computed Tomography
HGPIN	High grade prostatic intraepithelial neoplasia
MRI	Magnetic resonance imaging
PSA	Prostatic specific Antigen
PSAP	Prostatic specific acid phosphatase
TNM	Tumour, Node, metastasis
TUPP	Transurethral resection of the prostate
TUR	Transurethral resection
WHO	World Heath Organization
HMWCK	High molecular weight cytokeratin

CONCLUSIONS AND RECOMMENDATIONS

- The diagnosis of prostatic carcinoma in TRUS guided needle biopsies is typically established by use of traditional histologic parameters, including architecture, nuclear morphology, and absence of a basal cell layer.
- Because the visualization of basal cells is not always straightforward, immunohistochemical staining for antibodies to p63, can be applied to help confirm the diagnosis of small foci of carcinoma which would, by definition, lack expression of this marker.
- The use of AMACR along with p63 may help in the work-up of atypical acinar proliferations (ASL).
- The present study recommend the use of positive AMACR staining to convert an "atypical" diagnosis to cancer in cases that are highly suspicious for cancer on hematoxylin and eosin-stained section and basal cell markers are negative.
- AMACR is a sensitive marker of prostatic carcinoma, and its detection by immunohistochemistry staining in atypical prostatic lesions can be very useful in confirming an impression of adenocarcinoma.
- Only circumferential, diffuse or apical, granular, cytoplasmic staining of luminal cells that can be identified considered positive for AMACR.

- AMACR staining should be interpreted and used with caution, as a significant fraction of prostate cancer is negative for AMACR staining.
- From the diagnostic standpoint, it is important to recognize that positive AMACR staining does not equate to malignancy.
- As with any immunohistochemical reagent, *AMACR must therefore be used only in the context of strict morphologic correlation.*
- Further study is needed to follow up the patients with "atypical" diagnosis with either positive or negative AMACR staining, as this knowledge could have important implications on the management of the patients with "atypical" diagnosis.
- AMACR immunoreactivity cannot be used to distinguish prostatic carcinoma from HGPIN.
- The high rate of AMACR positivity in HGPIN suggests that such staining, if present in addition to a positive p63 immunostaining might be useful in confirming the diagnosis of HGPIN when the hematoxylin and eosin sections show features such as stratification, epithelial tufting, or cribriform or micropapillary structures suggestive of HGPIN, but the nuclear features are obscured by distortion, thick sectioning, or poor preservation.
- In the future, more sensitive and specific immunohistochemical markers are anticipated based on the

advances in our understanding of the molecular composition of prostatic cancer.

DISCUSSION

Prostate cancer is one of the most common malignant diseases for which health-care intervention is sought worldwide, and in many countries it is the most common noncutaneous malignant disease (Jani and Hellman, 2003). Attempts at the early detection of prostate cancer using serum prostate specific antigen levels and transrectal ultrasound have resulted in increased numbers of patients undergoing needle prostate biopsy. In addition, the number of biopsies on average from each patient has increased. Predictably, the advancements in the early detection of prostate cancer in the contemporary era has necessitated making diagnoses with seemingly smaller and smaller amounts of prostate cancer in needle biopsies (Beach *et al.*,2002).

The diagnosis of prostate cancer is usually readily made on morphological grounds by use of traditional histologic parameters, including architecture, nuclear features, and the presence or absence of a basal cell layer. However, in morphologically equivocal cases the histopathologist may have to resort to the use of immunohistochemistry to resolve the differential diagnosis (Varma and Jasani, 2005).

In recent years, there have been significant advances in prostatic cancer immunohistochemistry with the introduction of new markers such as **AMACR** and **p63** (Hameed *et al.*, 2005).

Some studies suggested that **AMACR** was uniformly and strongly positive in 97% (Jiang *et al.*, 2001) to 100 % (Luo *et al.*, 2002) of prostate cancer. The present study, however, found that

prostate cancer was positive for AMACR in only 86.5 % of cases. In addition, the AMACR staining in positive cases was not uniform, with the majority of cases exhibiting strong (45%) or moderate (37%) staining intensity, and only a minority of cases showing weak staining (5%). These results seem to reflect similar findings in other studies on prostate needle biopsies that demonstrated that AMACR was positive in only 62-90% of prostate cancer cases. Thus Beach *et al.* (2002) found AMACR positivity in 82% of prostatic carcinoma with 72% of 186 needle biopsies showed <50% positive tumor cells while only 32% of cases showed >50% tumor cell positivity. In the study by Zhou *et al.* (2004), 81.9% of the morphologically difficult prostatic cancers seen in consultation were AMACR positive but a significant proportion of these cases showed only weak immunoreactivity with focal apical granular staining and only 85% showed moderate or strong AMACR positivity. Magi-Galluzzi *et al.* (2003) observed AMACR positivity in 88% of 209 needle biopsies with small foci (<5% of core) of prostatic cancer. However they noted a wide variation in AMACR sensitivity in the material from different institutions, possibly related to differences in fixation and processing. Jiang *et al.* (2004) in a large multi-institutional study reported AMACR positivity in 97% of 454 cases of prostatic cancer and they demonstrated that AMACR was expressed at least focally in all cases of prostate carcinoma examined with 92% of cases showing diffuse expression with sensitivities varying from 96% to 100% among the five participating centers each representing a university in a state in the united states of America. The overall sensitivity and specificity of

AMACR as a prostate cancer marker was 97% (sensitivity) and 92% (specificity) in comparison to benign prostatic epithelium. This very high sensitivity and specificity can be explained by the large number of cases studied by Jiang and his colleagues (2004).

The present study showed high sensitivity for AMACR (87.5%) and specificity (81.25 %) and most of the benign foci were negative for AMACR expression.

There is a wide variation in AMACR sensitivity and specificity in the published literature, and this may reflect differences in primary antibody type (monoclonal or polyclonal) or concentration used, as well as fixation method, antigen retrieval protocol and other methodological factors. An additional source of variation in the reported sensitivity and specificity of AMACR appears to be related to the different approaches adopted for the interpretation of immunostaining. Some authors consider only moderate or strong staining intensity to be positive, since they found it difficult to distinguish reliably weak staining intensity from negative staining (Rubin *et al.*, 2002). In contrast, Beach *et al.* (2002) interpreted weak AMACR staining as positive, which could at least partially account for the relatively high proportion of benign needle biopsies (29%) that showed AMACR positivity in their study. The John Hopkins group interpret weak AMACR immunoreactivity in suspect glands are completely negative, while they consider even moderate staining in the suspect glands as negative if the background benign glands show a moderate level of AMACR immunoreactivity (Zhou *et al.*, 2003).

No statistical correlation between Gleason grade and AMACR immunoreactivity has reached in the present study, and AMACR was positive in different Gleason scores without predilection for a certain Gleason score and these results agree with those in literature as Jiang et al (2004) found that the relation between AMACR immunoreactivity and different Gleason scores was statistically insignificant, and that was also the results that Beach and his colleagues (2002) found, which agrees with the results of Molinie et al (2004).

This study also showed no statistical relation between AMACR positivity and the different stages of the radical prostatectomy specimens which implicates that AMACR is positive in prostatic carcinoma with no predilection to a specific stage.

The present study encountered two variants of prostatic carcinoma, two cases of prostatic ductal adenocarcinoma and four cases of small cell carcinoma and they both showed AMACR immunoreactivity, ductal (100%) and small cell (75%), which agrees with the results of Beach et al (2002) which revealed AMACR immunostaining in all variants, including ductal (100%) and mucinous (67%) with similar staining pattern as with conventional prostatic carcinoma.

The present study showed a small fraction of prostate cancer negative for AMACR staining (12.5 %). The diagnostic implication of this finding is that AMACR staining should be interpreted and used with caution. Hence, there is now a growing acceptance that AMACR can be negative in a subset of unequivocal carcinoma, especially specific morphological variants such as foamy prostate

cancer, pseudohyperplastic variant of prostatic carcinoma and atrophic carcinoma (Kunju *et al.*, 2005). Other studies have also demonstrated AMACR expression in nephrogenic adenoma, another benign mimic of prostate cancer (Gupta *et al.*, 2004).

Equally important, benign glands can occasionally be positive for AMACR staining, although in the majority they are only weakly positive and noncircumferential. In this study, 18.75% of benign cases were positive for AMACR, with all foci of incomplete basal cell hyperplasia negative for staining, two atrophic foci showed focal non circumferential weak staining, and the focus of adenosis lacked staining. This is in keeping with the results of Jiang *et al.* (2002) who found focal and weak staining of benign glands in 12% of cases, although small benign glandular proliferations such as atrophy were consistently negative. Zhou and his colleagues (2004), however, found a higher incidence (36.4 %), but this over expression may be due to that their cases were consultation cases; the majority of which were sent because of positive AMACR staining. They postulated that AMACR staining in these morphologically benign glands may represent the earliest preneoplastic change, although there are currently no data to support or refute this hypothesis. Hameed and his colleagues (2005) identified AMACR staining in 15.9% of benign prostatic glands, 4% of foci of atrophy, including a focus with strong staining. Most prior studies have found that foci of atrophy either completely lack AMACR staining or show weak partial staining in a small subset of cases (Jiang *et al.*, 2001; Beach *et al.*, 2002; Jiang *et al.*, 2002; Kunju *et al.*, 2003; Browne *et al.*, 2004; Farinola and

Epstein, 2004; Molinie *et al.*, 2004). Zhou *et al.*, (2002), however, found a much larger number of AMACR-positive foci of atrophy, which may be due to the fact that the authors, in contrast to the other studies, used a polyclonal anti-AMACR antibody. Because of the above findings, Hameed and his colleagues (2005) recommended view of AMACR immunostaining as an adjunct to histopathology, and never consider positive staining, regardless of intensity (particularly when partial), as diagnostic for prostatic carcinoma.

The only focus of adenosis (atypical adenomatous hyperplasia) encountered in this study was negative for AMACR. However, AMACR expression has been reported in 18-30% of cases of adenosis (Varma and Jasani, 2005), highlighting the need for caution when using AMACR to distinguish this type of lesion from prostatic cancer, particularly as the former often exhibits a discontinuous basal layer with some glands completely negative on basal cell marker immunostaining (Yang *et al.*, 2002). To complete the subject (although no cases were encountered in the present study), recently, two independent reports have collectively described AMACR positivity in 36 (53%) of 68 cases of nephrogenic adenoma, often with strong to moderate staining intensity resembling that observed in prostatic cancer (Gupta *et al.*, 2004; Skinnider *et al.*, 2004). Awareness of this potential diagnostic pitfall is particularly important as nephrogenic adenoma can morphologically mimic prostatic cancer and is generally negative for basal cell markers (Allan and Epstein, 2001). In the study by Gupta and his colleagues (2004), 26% of nephrogenic adenoma

cases were AMACR positive and HMWCK negative, an immunoprofile that would normally suggest prostatic cancer. To overcome this difficulty, in cases with morphological features suggestive of nephrogenic adenoma, the use of prostate specific antigen (PSA) immunostaining is recommended, as all the reported cases of nephrogenic adenoma have been noted to be PSA negative (Gilcrease *et al.*, 1998; Allan and Epstein, 2001; Gupta *et al.*, 2004; Skinnider *et al.*, 2004).

Ninety-four percent of cases of HGPIN showed positive staining with AMACR, with moderate to strong staining in approximately 70.5 % of the cases. These numbers are within the range described in the literature. Hameed *et al.* (2005) showed 95 % positive staining with AMACR alone, and 93% positive staining with AMACR using p63/AMACR antibody cocktail. Kunju and his colleagues (2005) reported 91 % positivity in contrast to Molinie and his colleagues (2004) which found AMACR immunoreactivity in 70% of cases with granular intracytoplasmic pattern and Beach *et al.(2002)* reported its expression in only 32% of cases of HGPIN. AMACR immunoreactivity cannot be used to distinguish prostatic carcinoma from HGPIN and the correlation was found to be statistically insignificant in the present study.

This high rate of AMACR staining in HGPIN indicates that one should be certain to exclude HGPIN before a diagnosis of carcinoma is made based on AMACR immunostaining alone when no basal cell marker is used, but this high rate of AMACR positivity suggests that such staining, if present in addition to a positive basal cell marker immunostaining, might be useful in