Efficacy of Supportive Histo-morphological Features in Prostate Cancer Diagnosis

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Abstract

Prostate cancer is a major disease of concern in adult males. Histological architectural heterogeneity has rendered challenges to diagnosis. Definitive diagnosis has been relied on Pathologist subjectivity. Major histologic features of infiltrative growth pattern, nuclear atypia and loss of basal cell on the prostate epithelium remain the most common features observed for in histopathological diagnosis. However benign mimicy of these features poses challenges to definitive diagnosis. Increasing frequency of supportive histologic features of prominent nucleoli, collagenous micronodules, perineural invasion, blue tinged mucinous secretions and intraluminal crystalloids which previously were unknown is making advancement in the diagnostic library as an additive protocol. In this study we sought to observe the frequencies of these supportive histologic features on a needle biopsy of prostate cancers. Mean age of diagnosis of prostate diseases was 69.15 ± 11.24 with incidence of prostate diseases as Benign Prostate Hyperplasia 148(50.6%), Prostate Carcinoma, 114(39.0%), Prostatitis 22(7.5%) and others 8(2.7%). In this inspection we found significant numbers of supportive histologic features on prostate cancer cases. Perineural invasion (38.1%), prominent nucleoli (34.3%), collagenous micro nodules (12.7%), intraluminal crystalloids (9.0%) and blue tinged mucinous secretions (6.0%) were explicitly expressed. The findings points to the consideration of these supportive histomorphological features in the histo-pathologic diagnosis of prostate carcinoma.

Keywords: Prostate carcinoma; Perineural invasion; Collagenous micronodules; Prominent nucleoli; Blue tinged mucinous secretions; Intraluminal crystalloids

Introduction

Prostate Cancer is regarded as the most common tumor of male adults. It is the considered as the second leading cause of male cancer deaths around the globe [1]. Epidemiological studies have shown increasing predominance amongst black populations [2,3]. Epidemiologic study in a Ghanaian population points to a 17.5% mortality rate describing it as an emerging disease [4]. Increasing trends of prostate cancer prevalence and mortality rates presents an alarming picture even in the midst of aggressive global campaign for healthy ageing.

Like many other malignancies; diagnosis of prostate cancer is initiated by a wide range of investigations from clinical presentation of subject to molecular investigations for prostate cancer expressive markers. However, histologic architectural presentation of the prostate gland tissue in defective conditions such as cancer is regarded as the commonest approach to diagnosis of prostate cancer [5]. In this common approach, normal histologic morphology is marked against defective changes on the prostate tissue. Consensus has been reached as posited by Totten et al., 1953, to base diagnosis of prostate cancer on three basic histologic features such as; absence of epithelial basal cell layer, glandular infiltration of tumor and variations in nuclear structure [6]. This general approach aided by the Gleason grading which measures the extent of differentiation of prostate epithelial tissue has been used widely albeit reported challenges.

Challenges have been reported to ensue due to the complexity in anatomical structure of the prostate gland which renders high rate un-specificity in targeted sampling [7,8]. The challenge is further compounded by the needle biopsy sample mostly used for diagnosis. Needle biopsy strand-like tissues present with a minimal focus of view which undermines extensive microscopic scanning for histologic features unlike the radical prostatectomy sample [9,10]. It thus permits the concealment of cardinal histologic features which can aid in diagnosis. Furthermore heterogeneity of prostate tumor histologic morphology calls for mimicry of prostate cancer to other prostate diseases [11,12]. Prostate diseases such as Atypical Adenomatous hyperplasia (AAH) is observed to present with similar morphologic picture like prostate cancer [13].

The aforementioned challenges have necessitated the need for other approaches that best provides definitive histologic diagnosis of prostate cancer. Predictions on the usefulness of other histologic features otherwise present on the prostate cancer tissue but infrequently observed for diagnosis have received attention [14]. Varma et al., have reported the presence of other infrequent histologic features and postulated it to be efficacious in confirming difficult diagnosis.

In low resource countries with continuing higher prevalence of prostate cancers but unable to afford somewhat an expensive confirmatory approaches such as molecular diagnostics; diagnosis is solely placed in the microscopists subjectivity on the conventional approach known in literature. It thus become very imperative to go along the lines of Varma et al., and many others in finding the prevalence of these infrequent histologic features on the prostate cancer tissue [14].

Establishment of the prevalence of these histologic features could serve to postulate the efficacy of these histologic features as adjunct to the cardinal features.

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Received March 22, 2014; Accepted May 29, 2014; Published September 17, 2014


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Methodology

H&E stained slides of prostate needle biopsies were retrieved from the archives of the Department of Pathology Komfo Anokye Teaching Hospital Kumasi from 2009-2011. All prostate cancer slides were reviewed for conventional tumor diagnostic features of; an infiltrative growth pattern, presence of nuclear atypia and absence of epithelial basal layer. An autopsy prostate tissue of a twenty five (25) year old male with no clinical history of prostate disease was collected, processed and stained with H & E and used as a negative control. The age of twenty five (25) was used against the backdrop of literature that prostate cancers are most common at after age forty (40) [1]. The prostate gland tissue thus as processed presented with clearly uniform fibromuscular stroma with clear lumen and fine nuclei arrangement (Figure 1).

We also observed for specific histologic features described as supportive criteria such as prominent nucleoli, perineural invasion, intraluminal crystalloids, blue tinged mucinous secretions and collagenous micro nodules. Histomorphology of these features were characterized as described in literature. The degree of prostate cancer epithelial differentiation was graded using the Gleason’s grading system. Grades were grouped into composite Gleason scores (GS) as; well (GS 2-4), moderately (GS 5-7) and poorly differentiated (GS 8-10). Graphpad prism 6 statistical software was used in the data analysis in the planned parameters of frequency distribution and descriptive statistics.

Results

Clinico-pathological data

The distribution of prostate diseases as diagnosed histologically from the archives is shown in table 1.0. Benign prostate hyperplasia (BPH) represented the common prostate disease in the study population with incidence of 50.6%, n=148 whiles prostate cancer (CaP) was 39.0%, n=114. Histological mean age of prostate diseases was 69.15 ± 11.24 with mean age of prostate cancer as 75.03 ± 10.17 (Table 1). Bonferroni’s post hoc analysis showed age exerting significant effect on all prostate diseases as shown in Figure 1 (**P ≤ 0.001). This demonstrates the risk of ageing to the development of prostate diseases.

Gleason grading pattern trends observed

We classified the degree of differentiations into well differentiated (GS 2-4), moderately differentiated (GS 5-7) and poorly differentiated (GS 8-10). Results of tumor differentiation classifications are shown in Figure 2. We conducted a correlative analysis of age with Gleason scores as shown in Figure 3.

Discussion

Prostate gland somewhat an insignificant organ of the male reproductive system presents with deleterious pathologies. Key amongst them is prostate cancer which is considered as the prominent cause of increasing male cancer-specific mortalities [1]. As common to most cancer diseases, prostate cancer presents with varied etiologic...
Table 1: Incidence and Histological Diagnostic Ages of Prostate Diseases Variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PN (%</th>
<th>Pi (%</th>
<th>CM (%)</th>
<th>IC (%)</th>
<th>IBTM (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>46(34.3)</td>
<td>51(38.1)</td>
<td>17(12.7)</td>
<td>12(9.0)</td>
<td>8(6.0)</td>
<td>-</td>
</tr>
<tr>
<td>Age(years)</td>
<td>77.46 ± 11.19</td>
<td>77 ± 8.78</td>
<td>79.71 ± 8.62</td>
<td>78.09 ± 4.42</td>
<td>84.63 ± 6.23</td>
<td>0.2604</td>
</tr>
<tr>
<td>Gleason</td>
<td>7.48 ± 1.53</td>
<td>7.63 ± 1.31</td>
<td>8.18 ± 1.07</td>
<td>8.55 ± 1.29</td>
<td>8.6 ± 1.27</td>
<td>0.0261</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean ± SD. PN: Prominent Nucleoli; PI: Perineural Invasion; CM: Collagenous Micronodules; IC: Intraluminal Crystalloids; IBTM: Intraluminal Blue Tinged Mucinous Secretion; N: sum total of each minor criterion observed in prostate cancer cases.

Table 2: Incidence of Supportive Features with Age and Gleason Score Correlate.

![Figure 2: Effect of Age of CA with other conditions of prostate.](image1)

![Figure 3: (A) Degree of differentiation of cancer. (B) Regression of GS on Age of CaP.](image2)
agents that concomitantly affects its specific diagnostic approaches and prognostic outcomes. Reliance has however been rested in the conventional histo-pathologic assessment for histologic diagnostic features but with challenges in benign mimicry. In the bid to resolving the difficulty that may arise in using the conventional approach we sought to demonstrate the presence and prevalence of other infrequent histologic features that may serve as adjunct to the histo-pathologic diagnostic protocol of prostate cancers. These supportive diagnostic features could be used as surrogate confirmatory indicators in low resourced settings without the advantage of the many time honored molecular approaches.

**Clinico-pathological finding**

In the period of study of 2009 to 2011, we observed 292 prostate needle biopsy slides with clinico-pathological outcome as shown in Table 1. Common to literature Benign Prostate Hyperplasia (BPH) was the most common prostate disease observed with an incidence of 67.02 ± 9.34 %. Literatures understudying the pathogenesis of prostate disease pattern have strongly predicted the risk of males to BPHs owing to the decreased production of testosterone in ageing which is deemed to maintain the normal growth of prostate gland [15]. Reduced production of testosterone thereby could lead to epithelial and stromal hyperplasia which results in the formation of nodules noted as BPH. The problem is exasperated with the linkage of BPH as a pre-malignant lesion as posited by Foster, 2000. From this assertion it becomes increasingly worrying for the study population with reported similar trends of high BPH prevalence by Gyasi-Sarpong et al. [16]. Though no direct linkage studies have been initiated by this researcher or researchers from this study population one could easily predict that the increasing prevalence of BPH could as well increase the prevalence rates of the much deleterious prostate cancer [17].

Ageing is considered as a time honored risk factor to prostate diseases though the exact role of ageing to prostate cancer pathogenesis remains under discussion. However it is generally observed that ageing may lead to variations in prostate gland vasculature as well as stromal morphology which may contribute to cellular neoplasia. The advent of Prostate Specific Antigen (PSA) has set an age index of forty years (40years) as risk to prostate cancer [17,18]. To us and many others this age specific risk index can be faulted owing to the false negative results of PSA screening [19]. We posit that if histo-pathologic diagnosis rendered as the most common confirmatory approach to prostate cancer diagnosis then age of incidence at histo-pathologic diagnosis could be a better predictor of age risk to prostate cancer. From Table 1, we show that a man is likely to be at risk to prostate disease at 69.15 ± 11.24 years contrary to PSA set age of 35 years. Specific to prostate cancer it was picked histologically at 75.03 ± 10.17 years. Interestingly we found out that the histologic age risk to CaP to be lower in high income study populations than low income study populations. Hennis et al., picked CaP histologically at age 50 years whiles Ezenwa et al., picked at age 65 years somehow consistent with our observation [20]. This inconsistency can be attributable to prostate disease illiteracy as well as inaccessibility to personnel and diagnostic equipment’s.

We found a direct correlation between ageing and degree of tumor differentiation ($r^2=0.28$, $\beta=0.07$, $p<0.001$; Figure 3B). Prostate tumors like many other tumors exhibit cross differentiation of the epithelial tissues a stage which affects management and prognostic outcomes [21]. Thus the extent of differentiation determines the management approach to the disease. Under the statistical stratification we found both moderately (GS 5-7) and poorly differentiated (GS 8-10) cancers amongst the highly aged population. It conforms to studies that ageing prostate epithelium is susceptible to medial fibrosis and intimal thickening of the arteries in the periphery of the gland which pre-mediate carcinogenesis [19,22]. This finding further substantiates our earlier claim of ageing as cardinal risk factor to prostate cancer.

In testing the hypothesis as posited by Kovi et al., and Otter et al., on the expressiveness of ageing on all prostate disease; we found significant expression on all (Figure 2; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) [19,23]. This affirms that indeed age again is a key predictor to prostate diseases. The increasing trends in ageing as observed can give an insight into the pathological sequence of prostate diseases. Thus, prostate diseases progress from less invasive prostatitis to prostate cancer as suggested by Foster (2000) [15]. For instance a subject of age 67.02 ± 9.34 years histologically confirmed as BPH if not managed could transform with an advancing age of 75.03 ± 10.17 years to prostate cancer. We also demonstrated statistically that it took a subject 10 years to progress to the next stage of prostate disease such as inflammation (prostatitis) to benign conditions (BPH).This however contradicts earlier studies by Crawford, (2003) which predicted the progressive age as 5 years.

We submit however that this information is only presumptive on prostate cancer etiology and pathogenesis. Specific time based monitoring of sequence of pathogenic progression of the diseases in molecular and tissue culture studies will be more appropriate.

**Histological architecture**

Prostate gland presents with a heterogeneous histological architecture same as its gross anatomy. The tissue as photomicrographically shown in Figure 1 as a negative control had stroma with secretory glandular cells with follicles and elongated canals which joined to form ducts. The follicles were supported by fine capillary plexus. The basal cells had a fine nuclear arrangement and normochromasia.

**Pattern of tumor differentiation**

Gleason grading system measures the extent of histologic tumor differentiation on the heterogeneous prostate stroma in a scale of 1-5. The score Gleason Score (GS) obtained by the summation of a primary less differentiated tumor to secondary more differentiated tumor were stratified as; well differentiated (GS 2-4), moderately differentiated (GS 5-7) and poorly differentiated (GS 6-10).

Well differentiated (GS 2-4) tumors consisted of well-formed glands with relatively simple contours, often rounded with uniform, tall, and columnar lining cells with pale to clear cytoplasm and basal dark nuclei are infrequently observed (fig 4A). Well differentiated tumors are generally uncommonly observed due to the late diagnosis of most prostate cancer cases with reported frequency of between 1-2% [24,25]. In this study we found 3(2.6%) cases with histologic picture synonymous to well differentiated tumors as shown in fig 4A. Our figure is also consistent with a similar black Nigerian population as reported by Ezenwa et al., 2008 (GS 2-4; 3(21.4%)) [20]. The slight shot above the generally known statistical range can be for by the dynamics in the study populations. Variations in resources and awareness to prostate disease with its early diagnostic approaches amongst different populations could account for this slight drift.

Again the notion by Epstein, that well differentiated tumors are most often mis-graded (down or upgraded) could also account for the variation. Epstein’s reports that a vast majority of tumors graded as Gleason score 2–4 on needle biopsy, when reviewed by experts in urologic pathology, are graded as Gleason scores 5–6 or higher [26]. Therefore inter-observer variations amongst various pathologists...
could account for the difference in incidence rates. We however did not study into the comparative grading errors amongst pathologist in this study. This notwithstanding we advise that attention be paid to diagnosing and selection of treatment protocol for well-differentiated (GS2-4) cancers since it is described by Cury et al., to invade prostatic capsule and hence progressive [8]. We also recommend a study into the histologic grading errors in prostate gland pathology.

Moderately differentiated (GS 5-7) are largely appreciative of malignancy with stromal separation of glands, infiltrative growth pattern and darker cytology due to cytoplasmic basophilia (Figure 4B). Gleason scores of 5-7 with embedded pattern 3 are the most common histologic grades of prostatic adenocarcinoma. We observed 61(53.5%) of moderately differentiated carcinomas in this study which is reflective of the general prevalence trend of Gleason score assessments [27,28]. Significantly GS 5-7 are easily recognizable owing its histologic presentation and pose no or little challenge to pathologist.

Histological architecture of GS 8-10 presented with a sheeted cell layer of epithelium with loss of basal cell layer, infiltrative pattern and nuclei anaplasia (Figure 4C). The nuclei atypia ranged from hypo and hyper chromatic as well as to few nucleomegaly. We observed 50(43.86%) of poorly differentiated (GS 8-10) carcinoma; an observation which is also consistent with the general trend of frequency of prostatic tissue degree of differentiation [20,29,30]. However, this study observed prevalence of 53.5% as higher to studies by Ezenwa et al., (2012) and King, (2000) is rather alarming [29,30]. Poorly differentiated tumors are predicted to have poor prognostic outcome with accompanying high mortality rates and makes this case of high reported values a greater concern [21]. It is however a good note that poorly differentiated carcinoma is easily recognized by pathologist owing to its unique sheeted histologic morphology. Our observation of similar trends in frequency of degree of differentiation is a major boost to histologic prostate cancer diagnosis as it enforces pathologist efforts to diagnosis. It is also informative to clinicians on the best treatment protocol to be selected. Gueron et al., also points out adverse effect of certain treatment outcomes on advanced prostate cancers such as Gleason grades 8-10 [31]. Clinicians must therefore take note of such effects of mortality and survival outcomes on certain treatment approaches and recommend the best approaches. In a subsequent study by this team of researchers; we will be looking at the rate of differentiation of the prostatic epithelium with the cell differentiation marker Ki 67 and p53 and correlate it on treatment procedures.

Another subject of interest was the finding of quite a similar frequency for Gleason score 7 and 8 respectively seen as; GS 7 (32, n=114) and GS 8 (33, n=114). This brings in the question and difficulty in summation complexity of Gleason grades 3, 4 and 5 in arriving as such scores as posed by Humphrey [27]. According to Humphrey various shades of grades exist for such grades which make its observation and summation difficult [27]. This position was further pointed out by Fine and Epstein, who on focusing on Gleason grade 4 encountered 18.1% (264 of 1,455) of cases with varying interpretation [10]. The same trend could be accounted for the observed similar frequency of Gleason score 7 and 8. To this effect we concur to the proposal by Humphrey, (2004) the inclusion of a tertiary grade in Gleason grading scheme. It is worthy of note also as a matter of reform to refrain from the practice of using the nomenclature of lumping such as mild, moderate and high in the reporting of prostate cancer Gleason grades. When such is used vital specific information on tumor histologic morphology is lost which can have implication on treatment and management procedures.

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![Figure 4: (A) Well Differentiated Tumor. (B) Moderately Differentiated Tumor. (C) Poorly Differentiated Tumor.](image-url)
Supportive histologic features observed

Histologic heterogeneity of the prostate epithelium renders difficult definitive diagnosis of prostate cancer. Heterogeneity of the conventional histologic features such as nuclei atypia, absence of basal cell layer and infiltrative malignant pattern also makes prostate cancer diagnosis indefinite. The resultant mimicry of benign to malignant vice-versa poses difficulty in diagnosing prostate cancer. In view of this Varma et al. and Algaba et al. have recommended the use of other histologic features to serve as supportive criteria in prostate cancer diagnosis [11,14]. For a low resource population with deficits in molecular confirmatory approaches, demonstration of these uncommon histologic features could serve as surrogate indicators in prostate cancer confirmatory histologic diagnosis.

Nuclear morphometry has played a significant role in prostate cancer diagnosis. Nuclear changes such as nucleomegaly, nucleolar prominence, nucleolar margination multiple nucleoli and prominent nucleoli have all been advocated for prostate cancer diagnosis [11]. The presence of prominent nucleoli as shown on Figure 5A has received much attention, but the debate lingers on the definitive predictive value of prominent nucleoli unlike multiple nucleoli which is considered to be more specific for cancer [32-34]. Iczkowski and Bostwick, in a study based on consultation material reported of 100% prominent nucleoli on prostate cancer cells. Epstein’s, study on prostate needle biopsies contradicted this study by finding as many as 24% of prostate cancers without prominent nucleoli. Varma et al. also found 25% of prominent nucleoli on benign prostates. In this study we found 34.3% of nucleoli with a larger size evidenced as prominent nucleoli on prostate cancer slides [14]. Indeed our finding is higher than aforementioned reports due to inconsistencies in pre-analytical procedures done by different histotechnologists. However, the presence of prominent nucleoli on prostate cancer specimen as observed and consistent with Varma et al., and Epstein’s study is suggestive of its notification and inclusion in the histologic prostate cancer diagnostic protocol [14,32]. Though we did not do comparative study with benign prostates we tend to walk with Epstein, in its in-absolute exclusivity to prostate cancers.

Figure 5: (A) Tumor Growth. (B) Collageneous Micronodules. (C) Intraluminal Crystalloids.
Perineural Invasion (PIN) is another histologic finding that has been widely recommended for inclusion in prostate cancer diagnosis on all needle biopsies. PIN was observed as the presence of prostate cancer tracking along or around a nerve within the perineural space (Figure 5B). Stroma of perineural sheath has been described to promote tumor growth by serving as a conduit enhancing extra prostatic tumor spread and has been the basis of extensive research on PIN as predictive and prognostic marker for prostate carcinoma [35,36]. However the advocacy of PIN as a definitive histologic feature of prostate carcinoma provides varying frequency trends. de la Taille et al. reports of a 24% PIN on needle biopsies while Bastacky et al. reports of 20% on prostate cancers. Humphrey, predicts a general prevalence of 11-37% of PIN on needle biopsies of malignant prostate tumors. We found 38.1% PIN on needle biopsies with cancer in this study [37]. The higher prevalence as observed in this study gives an indication of the necessity of PIN in histologic prostate cancer diagnosis. The presence of PIN on a prostate needle biopsy can thus be highly predictive of cancer if also combined with other histologic features.

Intraluminal crystalloids (IC) are distinct, brightly eosinophilic, refractile structures with varying shapes and sizes described first by Holmes, have been shown to be pathognomonic of prostate cancer. Epstein et al.’s observation of intraluminal crystalloids on atypical hyperplasia has rendered it unspecific for prostate cancer [13]. The prevalence of IC on prostate needle biopsies ranges from 10-64.5% according to Jensen et al., and Anton et al., [37,38]. We saw 12(9%) as shown in Figure 5C distinct, refractile and eosinophilic contents in the prostatic stromal lumen consistent with intraluminal crystalloids. The data is a little higher above the minimal value of the general prevalence rate of IC as presented by Anton et al., [37,38] but however far above Varma et al,’s, outcome of 40.6% [14,38]. Again the reason can be attributed to pathologist experience as well as variations in tissue processing and staining techniques. The finding of intraluminal crystalloids on prostate cancer specimen is suggestive of its inclusion in the prostate cancer diagnostic library.

Another minor histologic feature forward to be diagnostic for prostate cancer is collagenous micro nodules. Collagenous micro nodules (CM) also called mucinous fibroplasia as shown in Figure 5D presented with aggregates of nodular paucicellular eosinophilic fibrillar stroma. It was seen as a delicate, loose fibrous tissue with ingrowth of fibroblasts and focal nodular hyalinization of mucinous secretions. We examined 8(6%) of CM on needle biopsies a figure we find as significant to prostate cancer diagnosis. Bostwick et al. found 0.6% of CM on needle biopsies and 12.7% on prostatectomies and none in benign glands. Similarly Leroy et al. and Varma et al. [39,40] found 1% and 2% respectively of CM on needle biopsies [39,40]. Increased observed trend of CM in the study is suggestive of its relevant predictor to histologic cancer diagnosis. Sufficient to say that its presence does not solely exclude histologic prostate cancer diagnosis.

The secretory luminal layer of prostate epithelium secreted mucinous contents demonstrable on H&E staining. It is rendered more specific for malignancy due to its 100% specificity and 52% sensitivity as well as its absence on benign glands as predicted by Humphrey, (2003) and Epstein, [32,41]. We observed 17(12.7%) of Blue Tinged Mucinous (BTM) secretions (Figure 5E) on prostate cancers as against Varma et al.’s, 78(52%) [14]. Observation of BTM is dependent on the quality of staining which also accounts for the striking differences. To achieve effective outcome meticulous effort must be taken in stain preparation and procedure.

Conclusion

In evaluating histologic architecture of prostate carcinoma we found Benign Prostate Hyperplasia to be predominant prostate disease even in the light of current public health awareness. We have shown the risk of ageing to prostate diseases with mean age of prostate cancer as 75.03 ± 10.17 years. We observed moderately differentiated tumors (GS 2-7) as the predominant category of tumor differentiation and that ageing increases significantly with the degree of spread (p value <.001 and r²=.28). We on this premise postulate that both proliferative activity and invasiveness increases from benign to malignant in the spectrum of prostatic lesions. We thus suggest an agressive public health education on effective healthy ageing. We have also shown the presence of minor histologic features prominent nucleoli, perineural invasion, collagenous micronodules, intraluminal crystalloids and blue tinged mucinuous secretions on prostate needle biopsies. By this study we point out that it is worthwhile to report on supportive histologic features and be adopted as a diagnostic policy; the inclusion of supportive/minor histologic features to the conventional major criteria used in prostate carcinoma diagnosis.

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