Polyclonal Anti-PSA Is More Sensitive but Less Specific Than Monoclonal Anti-PSA

Implications for Diagnostic Prostatic Pathology

Murali Varma, FRCPATH,1 Meleri Morgan, MBBS,1 Bharat Jasani, FRCPATH,1 Pheroze Tamboli, MD,2 and Mahul B. Amin, MD3

Key Words: Prostate-specific antigen; Immunohistochemistry; Prostate; Seminal vesicles; Sensitivity; Specificity

Abstract

Prostate-specific antigen (PSA) production by nonprostatic tissues has been reported, casting doubts on its specificity. The immunohistochemical relative specificity and sensitivity of PSA expression using monoclonal and polyclonal anti-PSA was analyzed on 60 prostate carcinomas, 40 normal seminal vesicles, and 310 nonprostatic tumors. All nonprostatic tumors proved negative with both antibodies. However, 13 (32%) seminal vesicles showed immunoreactivity with polyclonal anti-PSA, but none showed immunoreactivity with the monoclonal antibody. The sensitivity of the 2 antibodies for prostate cancer varied with tumor grade. In Gleason pattern 3, both antibodies showed diffuse immunostaining in all cases. In Gleason pattern 5, polyclonal anti-PSA showed diffuse (>95%) tumor cell positivity in 18 cases (90%), while with the monoclonal antibody, 7 cases (35%) showed only focal (<10%) tumor cell immunoreactivity. Thus, monoclonal anti-PSA seems to be useful in small gland proliferations in which the differential diagnosis includes seminal vesicle, while for poorly differentiated neoplasms, polyclonal anti-PSA is considered superior. Sections of high-grade prostate cancer should be included as positive controls for PSA immunostaining.

Immunohistochemical analysis using antibodies directed against prostate-specific antigen (PSA), a secretory product of prostatic epithelium, is widely used to establish the prostatic origin of metastatic adenocarcinoma in diagnostic histopathology practice. This has important clinical relevance, as the confirmation of prostatic origin would result in the patient being administered specific treatment, which is hormonal manipulation in most instances. PSA also is useful to distinguish seminal vesicle epithelium from prostatic cancer in needle biopsy specimens, a differential diagnosis of small acinar proliferation that is complicated further by the fact that pigment resembling that of the seminal vesicle may be seen in benign and malignant prostatic epithelium.1 PSA also is used to distinguish prostatic adenocarcinomas from other nonprostatic small acinar proliferations or normal histoanatomic variants such as Cowper glands, nephrogenic adenoma, and hyperplastic mesonephric remnants, as well as adenocarcinomas of rectum, urethra, and urinary bladder, which may have very similar histologic features and manifest in a pelvic location.

The use of PSA immunohistochemical analysis in the aforementioned clinical settings is based on the premise that PSA is specific for prostatic origin.2 However, there have been several individual case reports and small series documenting PSA immunoreactivity in a variety of nonprostatic tumors, including salivary gland neoplasms, carcinoma of the breast, urothelial (transitional cell) carcinoma, adenocarcinoma of the urinary bladder, colonic adenocarcinoma, and lung carcinoma.3-7 PSA immunoreactivity also has been reported in benign seminal vesicle epithelium.8,9

PSA immunoreactivity can be demonstrated using polyclonal and monoclonal antibodies that may differ in their
sensitivity and specificity for PSA. Traditionally, polyclonal anti-PSA is used widely in the United States, while several major centers in the United Kingdom use the monoclonal antibody. However, this choice of antibody type does not seem to be based on any detailed comparison of the 2 antibodies. Previous studies evaluating PSA immunohistochemical analysis generally have focused on either the sensitivity or the specificity of immunostaining, but not both together. However, a very specific technique may lack sensitivity, while improvements in sensitivity may be at the expense of specificity.

The aim of the present study was to systematically analyze the immunohistochemical specificity and sensitivity of PSA using both monoclonal and polyclonal anti-PSA applied with a contemporary antigen-retrieval method on a large series of cases spanning the range of prostatic carcinoma differentiation and in a diverse group of nonprostatic tissues, including those with reported cross-reactivity for PSA and tumors likely to be in the differential diagnosis of prostate cancer such as carcinoid tumors and urothelial carcinoma.

Materials and Methods

Routinely formalin-fixed and paraffin-embedded tissue sections from 60 prostate carcinomas, 40 benign seminal vesicles from radical prostatectomy specimens, and 310 nonprostatic tumors were obtained retrospectively from the histology files of the University Hospital of Wales, Cardiff; Emory University Hospital, Atlanta, GA; and the University of Texas M.D. Anderson Cancer Center, Houston.

Cases of prostate carcinoma were chosen such that a range of Gleason scores most likely to result in clinically significant prostate cancer were represented: 20 each with Gleason score 6 (3 + 3) and Gleason score 7 to 8 (3 + 4, 4 + 3, or 4 + 4) from radical prostatectomy specimens and 20 with Gleason score 10 from transurethral resections of the prostate. One representative section was selected from each individual case.

The 310 nonprostatic tumors included 40 breast adenocarcinomas (grade 1, 10; grade 2, 20; and grade 3, 10; using the Elston modification of the Bloom and Richardson grading system), 40 lung adenocarcinomas (20 each, well- and poorly differentiated), 40 colonic adenocarcinomas (20 each from the right side and the left side of the colon), 40 renal cell carcinomas (20 each, Fuhrman grade 1-2 and grade 3-4), 40 urothelial carcinomas (20 each, World Health Organization grade 1 and grade 3), 30 glandular lesions of the urinary bladder (urothelial carcinomas with glandular differentiation, 20; primary bladder adenocarcinoma, 10), 40 carcinoid tumors (20 each, lung and gastrointestinal), and 40 salivary gland tumors (20 each, pleomorphic salivary adenoma and salivary gland carcinoma types).

The H&E-stained slides of all cases were reviewed to confirm the diagnosis and grade and to ensure that representative tissue (well-fixed and nonnecrotic) was selected for immunostaining.

Tissue sections from each selected paraffin block were cut on frosted slides and immunostained using monoclonal anti-PSA (clone ER/PR8, DAKO, Ely, England; dilution of 1:160 applied for 45 minutes at room temperature) and polyclonal anti-PSA (catalog number, A0562, DAKO; dilution of 1:12,800 applied for 45 minutes at room temperature) with appropriate positive and negative controls. All cases were immunostained with both antibodies after microwave pretreatment (20 minutes in a 10-mmol/L concentration of EDTA, pH 7.0). The detection system used was the Vector Elite Universal system (Vector Laboratories, Burlingame, CA) with dianminobenzidine (Biogenex, San Ramon, CA) as the chromogen.

Immunohistochemical staining was assessed independently by 2 pathologists (M.V. and M.M.). The percentage of relevant cells staining positive and the intensity of staining (weak or strong) was recorded for each case. In prostate cancers with Gleason score 7, scoring was based only on the areas of pattern 4. Differences in scoring were resolved by subsequent simultaneous review and discussion with a third pathologist (B.J.) until consensus was reached.

Results

The immunostaining patterns of prostate adenocarcinoma using monoclonal and polyclonal anti-PSA are summarized in Table 1.

In Gleason pattern 3 prostate cancer, immunostaining with both antibodies was similar with diffuse PSA expression (>95% of tumor cells positive) in all cases Image 1. Strong immunostaining in more than 75% of cells was present with each antibody type in 19 cases (95%). In Gleason pattern 4 tumor, polyclonal antibody was more sensitive, with diffuse immunoreactivity (>95% cells positive) in 19 (95%) of 20 cases compared with 14 (70%) with monoclonal anti-PSA. Strong immunostaining in more than 75% of cells was observed in 18 (90%) of 20 cases with the polyclonal antibody and in 11 (55%) with the monoclonal antibody.

The difference between polyclonal and monoclonal anti-PSA was most marked in Gleason pattern 5 tumor Image 2. Monoclonal antibody was not sensitive in these tumors, with only 2 (10%) of 20 cases showing diffuse positivity and immunoreactivity in fewer than 10% of cells in 7 (35%) cases. Although none of the cases were entirely negative, 6
(30%) of 20 cases had chips from transurethral resections of the prostate with tumor cells totally negative for PSA (1-11 [median, 2.5] of 5-29 [median, 8.5] chips with tumor). Of 20 cases, 15 (75%) had chips with fewer than 5% of the tumor cells positive (8%-96% of chips; median, 50%). Polyclonal anti-PSA was more sensitive, with 18 (90%) of 20 Gleason pattern 5 tumors showing diffuse positivity and none of the cases showing fewer than 25% cells positive. None of the chips showed fewer than 25% positive cells using the polyclonal antibody. Diffuse staining was observed even in chips with cautery artifact.

All 310 nonprostatic tumors studied were negative with both monoclonal and polyclonal anti-PSA. However, with polyclonal anti-PSA, diffuse strong immunoreactivity was present along the luminal aspect of nonneoplastic salivary ducts in 27 (90%) of 30 salivary gland tumors in which nonneoplastic salivary glandular tissue was represented. No immunoreactivity was observed in these nonneoplastic ducts with monoclonal anti-PSA. Of 40 sections of seminal vesicle, 13 (32%) showed cytoplasmic immunoreactivity with polyclonal anti-PSA (1%-40% of cells positive; mean, 15.5%). The intensity of immunoreactivity was strong in 8 (62%) of these cases (3%-15% of cells; mean, 8.5%). All sections of seminal vesicle were negative with monoclonal anti-PSA Image 3I.

**Discussion**

Conflicting views have been expressed about the tissue specificity of PSA. While PSA generally has been considered specific for prostatic origin, more recently Levesque et al\(^5\) described PSA as “a relatively ubiquitous glycoprotein…perhaps a growth factor.” In our experience, PSA as

**Table 1** Distribution of Prostate-Specific Antigen Immunoreactivity in Prostate Cancer, According to the Grade of the Tumor and the Type of Antibody Used*  

<table>
<thead>
<tr>
<th>Gleason Pattern</th>
<th>Antibody Type</th>
<th>0</th>
<th>&lt;5</th>
<th>5-9</th>
<th>10-49</th>
<th>50-74</th>
<th>75-95</th>
<th>&gt;95</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (n = 20)</td>
<td>Monoclonal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Polyclonal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>4 (n = 20)</td>
<td>Monoclonal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Polyclonal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>95</td>
</tr>
<tr>
<td>5 (n = 20)</td>
<td>Monoclonal</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Polyclonal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>18</td>
<td>90</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) of cases.
detected by immunohistochemical analysis is very tissue-specific, as we did not find PSA immunoreactivity in any of the 310 nonprostatic tumors studied using monoclonal and polyclonal anti-PSA with heat-induced epitope retrieval (HIER). In view of the molecular biologic evidence of PSA production by nonprostatic tissues, it is likely that PSA is produced by tissues other than the prostate gland but at levels of expression too low to be detected by even the highly sensitive immunohistochemical techniques used at present. Our results are different from those of previous studies that showed immunohistochemical expression of PSA in some nonprostatic tumors. This is probably related to technical differences such as the source of the polyclonal antibody used. It has been suggested that at least 1 batch of polyclonal anti-PSA that had been used in some of the studies may have cross-reacted with cytokeratins.

While we did not find PSA expression in any of the nonprostatic tumors studied, notably urothelial carcinoma,
Varma et al / PSA IMMUNOHISTOCHEMICAL ANALYSIS

Adenocarcinoma of the urinary bladder, and carcinoid tumors, we did observe PSA positivity in the ductal epithelium of nonneoplastic salivary glands and in almost one third of the seminal vesicles with polyclonal but not monoclonal anti-PSA. These findings are similar to those reported in previous studies.\(^3,8,9\) PSA immunoreactivity in benign salivary ductal epithelium is interesting but of little clinical significance. In contrast, distinction of prostatic adenocarcinoma from glands of the seminal vesicle is important in 2 different settings. In prostate needle biopsy specimens, the glands of the seminal vesicle may mimic prostatic adenocarcinoma owing to the small acinar histologic features and nuclear atypia resulting in a possible false-positive diagnosis of cancer. In radical prostatectomies, rarely the peripheral budding and outpouchings of the seminal vesicle epithelium results in small acinar morphologic features with an infiltrative character such that carcinomatous involvement of the seminal vesicle becomes a serious consideration. Immunohistochemical analysis may be necessary to resolve this problem that was emphasized in the third series of the Armed Forces Institute of Pathology fascicle on the prostate and the seminal vesicle.\(^13\) However, to avoid misdiagnosing or overstaging prostate cancer, it is important to recognize that PSA expression may be detected in the seminal vesicle epithelium when polyclonal antibody is used. Possible explanations for PSA expression in the seminal vesicle include low-level aberrant expression demonstrated only by the more sensitive polyclonal antibody, nonspecific cross-reaction of polyclonal anti-PSA with some other antigen in seminal vesicle epithelium, or retrograde movement of PSA from prostatic ducts into the seminal vesicle.

Like previous studies, we found that PSA expression varies with the grade of prostate cancer.\(^14-16\) The percentage of positive cells and intensity of immunostaining decreased progressively with increasing tumor grade, especially with monoclonal anti-PSA. This may reflect heterogeneity in poorly differentiated cancer and may explain at least in part the relatively low serum PSA levels observed in some cases of poorly differentiated prostate cancer.

Polyclonal anti-PSA generally was substantially more sensitive than the monoclonal antibody in the diagnosis of prostate cancer. In Gleason pattern 3 tumor, both antibodies were very sensitive with diffuse immunoreactivity. However, the intensity of immunostaining was greater with the polyclonal antibody. The difference between the 2 antibodies was most marked in the high-grade tumors. With the monoclonal antibody, in Gleason pattern 5 tumor, diffuse staining (>95% cells positive) was seen only in 10% of cases (2/20), while fewer than 10% cells were positive in 35% of cases (7/20).

Although none of the cases were entirely immunonegative, chips with tumor totally negative for PSA were present in more than one third of cases and could be a potential source of false-negative immunohistochemical results. In contrast, polyclonal anti-PSA was highly sensitive even in Gleason pattern 5 tumors, with diffuse staining apparent in 90% of cases (18/20). Thus, differences in sensitivity of PSA immunostaining techniques may become apparent only when poorly differentiated prostate cancers are studied. Hence, we recommend that sections of high-grade (Gleason 5 + 5 = 10) prostate carcinoma also should be included as positive controls in PSA immunohistochemical analysis.

The sensitivity of PSA immunohistochemical analysis in the present study using polyclonal antibody was substantially higher than that reported in the literature.\(^\text{Table 2}\). Of the 95 cases of poorly differentiated prostate carcinoma in the 4 studies that used polyclonal anti-PSA, 7 (7%) totally lacked PSA immunostaining. In the present study, using polyclonal anti-PSA, more than 25% of tumor cells were positive in all poorly differentiated prostate cancers studied. The higher sensitivity may be related to the use of HIER in contrast with the older studies that predated the advent of this powerful technique.\(^19\)

The high sensitivity for prostate adenocarcinoma observed in our study using polyclonal anti-PSA with microwave HIER might be clinically significant. It has been generally accepted that while PSA positivity in a poorly differentiated carcinoma establishes prostatic origin, lack of PSA immunostaining does not rule out prostate carcinoma.

\begin{table}[h]
\centering
\caption{Prostate-Specific Antigen Immunoreactivity in Poorly Differentiated Prostate Cancer: A Review of the Literature*}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Reference and Year & No. of Cases & Antibody & Pretreatment & 0 & <5 & <25 & <50 \\
\hline
Stein et al.\(^\text{14} 1982\) & 7 & Polyclonal & None & 29 & NA & NA & NA \\
Ellis et al.\(^\text{15} 1984\) & 20 & Polyclonal & None & 5 & 25 & 25 & 25 \\
Svanholm\(^\text{16} 1986\) & 42 & Polyclonal & None & 5 & NA & NA & NA \\
Keillor and Aterman.\(^\text{17} 1987\) & 20 & NA & Trypsin & 10 & NA & NA & NA \\
Cho and Epstein.\(^\text{18} 1987\) & 26 & Polyclonal & None & 8 & 20 & 48 & 48 \\
Present study & 20 & Polyclonal & Microwave & 0 & 0 & 0 & 5 \\
\hline
\end{tabular}
\end{table}

NA, data not available.
* Data are given as percentage of cases.
as PSA expression is reduced in poorly differentiated prostate cancer. However, according to the present study, negative immunostaining in a poorly differentiated carcinoma using polyclonal anti-PSA with HIER would make a prostatic origin very unlikely.

PSA immunohistochemical analysis is confirmed to be highly specific and sensitive for establishing the prostatic origin of a tumor. In small gland proliferations in which the differential diagnosis includes the seminal vesicle, the use of monoclonal anti-PSA is recommended in view of false-positive staining of seminal vesicle epithelium with the polyclonal antibody. In metastatic prostate cancer, which is most often Gleason pattern 4 or 5 and frequently present in limited diagnostic material, polyclonal anti-PSA is considered superior to the monoclonal antibody. We recommend that sections of poorly differentiated prostate cancer should be included as positive controls, as these would provide a more stringent check of the sensitivity of PSA immunohistochemical analysis.

References