Clinical Considerations after a Negative Prostate Biopsy

E David Crawford¹, Neal Shore², Matthew Cooperberg³, Marc D’Alpaos⁴ and Francisco G La Rosa⁵

¹University of Colorado Health Science Center, Aurora, CO, USA
²Carolina Urologic Research Center, Atlantic Urology Clinics, Myrtle Beach, SC, USA
³University of California, San Francisco, San Francisco, CA, USA
⁴University of California Davis Comprehensive Cancer Center, Sacramento, CA, USA
⁵University of Colorado Cancer Center, Denver, CO, USA

*Corresponding author: E David Crawford, University of Colorado, Denver, Aurora, CO-80045, USA, Tel: 720-848-0195; Email: edc@edavidcrawford.com

Keywords: Prostate cancer; Repeat biopsy; Assay; Biomarker

Introduction

Approximately 1.3 million prostate biopsy procedures are performed annually [1] in the United States, despite only 220,800 new PCA diagnoses being expected in 2016 [2]. These biopsy procedures are most often instigated due to elevated prostate-specific antigen (PSA) levels to allow PCs to be discovered at an early stage.

The standard 12-core TRUS biopsy schema, which includes the standard sextant as well as a lateral sextant scheme [3], even when assisted by mpMRI [4], is not infallible. With this approach, actually less than 1% of the entire gland is sampled, providing limited histopathological analysis and resulting in a significant false-negative cancer-detection rate [5,6].

Studies have shown that nearly 60% to 70% of biopsies fail to detect PCs in men who were thought to harbor the disease [1,7]. This means that over 1 million men receive a negative biopsy reading but are still at risk of having a PCA for the reasons stated above. Approximately 25% of men with an initial benign biopsy result will have PCs detected on a subsequent biopsy [8]. In addition to the obvious direct clinical costs, false-negative prostate biopsies, i.e. biopsies classified as benign but containing adenocarcinoma or atypical suspicious glands, are often associated with additional medical and non-medical costs such as socio-behavioral distress, psychological impact [9], and increased medical utilization [10].

Finally, the biopsy procedure itself is invasive, puts the patient at risk for complications, and is subject to significant sampling errors [11].

Given that the traditional diagnostic pathway involves a nonspecific PSA blood test at the beginning, which then could trigger an invasive prostate biopsy, the risks and benefits of prostate biopsy are germane to the ongoing debate about early detection of PCs [12].

One of the criticisms of early detection with PSA relates to over-detection and overtreatment [13]. Indolent vs. aggressive cancer is not detected and therefore PCs are treated aggressively when it may not need to be [13]. In addition to high financial costs and subsequent side effects of unnecessary aggressive treatment, early detection can also result in repetitive biopsies (leading to high costs and morbidities).

Challenges in the Repeat Biopsy Setting

It is obvious that a negative biopsy does not preclude a diagnosis of PCs on subsequent biopsies. Of patients with suspicious PSA findings and a negative initial biopsy, 43% will undergo a repeat biopsy within 3 years of the initial biopsy [14]. Additionally, many will continue on this trajectory, with the rates of a third and fourth biopsy after a previous negative biopsy being similar to the initial repeat biopsy rate in men with elevated PSA levels [14].

Since repeat biopsies are similarly invasive procedures as initial biopsies, these too lead to an increased risk of infection, particularly: drug-resistant Escherichia coli; hospitalization; discomfort; anxiety; complications such as urinary tract infection (UTI), epididymitis, orchitis, prostatitis, and sepsis; and morbidities such as rectal bleeding, hematuria, vasovagal episodes, fever, hematospermia, and dysuria [12,15,16]. In the ProtecT study, 20% of men reported that they would consider a future biopsy a "moderate or major problem" [17].
Patients with repeat biopsies are sometimes more prone to drug resistance after being exposed to antibiotics. For example, patients undergoing a TRUS biopsy are at risk for developing prostatitis, which may be prevented with fluoroquinolones. However, about 50% of post-biopsy infections are resistant to fluoroquinolone and many are also resistant to other antibiotics [18,19]. Similarly, patients with prior use of fluoroquinolone are at risk of sepsis following a biopsy, due to drug resistance [20]. Undergoing repeat biopsies, therefore, puts the patient at risk for developing drug resistance, which could lead to impairing their immune systems in case of future sickness.

As with initial biopsies, repeat biopsies are associated with sampling error, impact on patient outcomes, risk of infections, and clinical complications. Sampling error is an inherent and well-documented issue with false-negative rates of prostate biopsy procedures reported as high as 25% to 35% [21]. Multiple prostate biopsies also increase the number of serious infections and hospitalizations [22].

Two major types of biopsy procedures are used to obtain prostate tissue for diagnosis of PCa: transperineal and transrectal. The main differences between these two approaches are in the route of puncture, site of puncture and the TRUS transducer [23]. While the two techniques have similar detection rates [24], studies have demonstrated that the transperineal approach tends to have a lower risk of infection compared with the transrectal approach [25].

Accurate diagnosis in patients with negative initial findings of biopsy is often complicated by continued elevation of serum PSA levels, resulting in a distinct management challenge [26]. While PSA has a role at the beginning of the PCa diagnostic pathway, its specificity for early intervention in repeat biopsy is unsatisfactory [27,28].

Persistent increases in PSA after a second negative biopsy presents a difficult clinical challenge because the detection rate of prostate biopsy after a first and second negative biopsy set is dramatically low, at about 10% [5]. For patients with an initial negative biopsy but with persistently elevated or rising PSA, abnormal DRE, or other risk factors indicative of missed PCa, few options are currently available to guide an urologist in determining whether or when additional biopsies are warranted.

Given the concerns about over-diagnosis and over-treatment of PCa, experts recommend that patients and their physicians (i.e., urologist, radiation oncologist, medical oncologist, primary care physician) consider active surveillance based on careful consideration of the patient's prostate cancer risk profile, age, and health [29].

Among men diagnosed with cancer on prostate biopsy, the National Comprehensive Cancer Network (NCCN) guidelines for prostate cancer detection [11] do not recommend routine repeat biopsy, except in certain circumstances, e.g., where the patient is suspected to harbor a more aggressive cancer than was evident on the initial biopsy and is otherwise a candidate for active surveillance as outlined in the NCCN guidelines for prostate cancer treatment [29].

As previously mentioned, significant costs are attributed to unnecessary biopsy procedures [30,31]. Approximately $4.4 billion is spent annually on screening, diagnosing and staging, and an additional $9.9 billion annually on treatment of PCa patients, totaling nearly $15 billion per year on PCa in the US alone [30-32]. Given that a considerable number of men have secondary procedures with increased risks, it is inherent to try and find ways to avoid as many unnecessary biopsies as possible.

Clinical Tools to Enhance Detection and Avoid Unnecessary Repeat Biopsies

It is well established that a negative prostate biopsy does not preclude a diagnosis of PCa on subsequent biopsy. As such, selecting the appropriate candidate for repeat biopsy remains a challenging clinical dilemma [33].

Traditional risk-stratification tools in the repeat biopsy setting such as PSA level, PSA velocity (PSAV), PSA density (PSAD), %free PSA (fPSA), and presence of histological features such as “multifocal” high-grade prostatic intraepithelial neoplasia (HGPIN) have limited correlation with cancer diagnosis or with the clinical significance of the disease (Figure 1) [34].

Thus, refining patient selection for biopsies by using biomarker tests to decrease unnecessary biopsies and increase the specificity of cancer detection, without missing a substantial number of higher-grade (Gleason ≥7) cancers, is warranted [33].

Following a benign biopsy result, the NCCN advises clinicians to consider a biomarker assay and repeat the prostate biopsy based on the assessed risk [11]. Biomarkers that are mentioned in the NCCN guidelines with regards to improving specificity in the post-biopsy state include PCA3, PHI, ConfirmMDx, and 4Kscore [11].

These tests should be considered in patients thought to be at higher risk despite a negative prostate biopsy:

- **PCA3:** Prostate Cancer Antigen 3 (PCA3) is a urinary biomarker of PCa that shows superior results to PSA scores in determining outcomes after repeat biopsies [35,36]. PCA3 appears to have utility in determining which patients should undergo a repeat biopsy because of a higher specificity for PCa compared to PSA [37-40]. Results from the NCI Early Detection Research Network (EDRN) validation study demonstrated a PPV of 80% for the detection of any cancer in the initial biopsy setting (at a score >60) and Negative Predictive Value (NPV) of 88% in the repeat biopsy setting (at a score <20) [41]. Applying PCA3 in the repeat biopsy setting would reduce the number of biopsies by nearly half, and only 3% of men who would have been
advised against a biopsy would harbor high-grade disease (Table 1) [42].

<table>
<thead>
<tr>
<th>Assay</th>
<th>Biomarkers</th>
<th>Key Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA3 (qPCR)</td>
<td>PCA3</td>
<td>•Indicated in men ≥ 50 year old to determine which patients should undergo a repeat biopsy because of a higher specificity for PCa compared to PSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Reduces re-biopsy rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Extensive validation</td>
</tr>
<tr>
<td>PHI</td>
<td>tPSA, fPSA and proPSA</td>
<td>•Distinguishing PCa from benign prostatic conditions</td>
</tr>
<tr>
<td>(Immunoassay)</td>
<td></td>
<td>•For use in men with serum PSA 4 ng/ml to 10 ng/ml and negative DRE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Increases specificity for detecting aggressive PCa (Gleason Score ≥ 7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Extensive validation</td>
</tr>
<tr>
<td>ConfirmMDx</td>
<td>GSTP1, APC, and RASSF1 methylation</td>
<td>•Aids in the reduction of unnecessary repeat biopsies</td>
</tr>
<tr>
<td>(Epigenetic assay)</td>
<td></td>
<td>•Independent predictor of patient outcomes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•NPV of 96% for clinically significant prostate cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Extensive validation</td>
</tr>
<tr>
<td>4Kscore</td>
<td>PSA, fPSA, intact PSA, HK-2</td>
<td>•Differentiate clinically insignificant tumors from aggressive PCa</td>
</tr>
<tr>
<td>(Immunoassay)</td>
<td></td>
<td>•Increases probability of detecting aggressive disease (Gleason Score ≥ 7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Decreases unnecessary secondary biopsies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Applicable regardless of age, PSA, or clinical findings</td>
</tr>
<tr>
<td></td>
<td></td>
<td>•Extensive validation</td>
</tr>
</tbody>
</table>

Table 1: Biomarkers mentioned in the NCCN guidelines for improving specificity in the post-biopsy state.

**PHI:** The PHI test represents a combination of tPSA, fPSA, and a subcategory of free PSA called pro-PSA [43-45]. A multi-center study demonstrated that PHI exceeded the specificity and AUC (0.70) of PSA and %fPSA for the 2 ng/ml to 10 ng/ml PSA range (at 80% to 95% sensitivity) [46]. Increasing PHI was associated with a 4.7-fold increased risk of PCa and 1.61-fold increased risk of Gleason ≥ 7 disease upon biopsy [46]. Additionally, the AUC for PHI (0.72) exceeded that of %fPSA (0.670) in discriminating between PCa with Gleason ≥ 4+3 vs. lower grade disease or negative biopsies [46]. In 2012 the FDA approved PHI for use in men with serum PSA between 4 ng/ml to 10 ng/ml.

**ConfirmMDx:** ConfirmMDx is a tissue-based, multiplex epigenetic assay that addresses false-negative biopsy concerns by helping to identify men who may forego an unnecessary repeat biopsy. In addition to its utility in reducing unnecessary biopsies, the assay has been shown to be a significant independent predictor of patient outcomes [47-49]. Most prostate tumors have epigenetic DNA-methylation aberrations, which display a field effect that can be observed in histologically normal-appearing surrounding tissue. The ConfirmMDx genetic assay detects DNA methylation of three PCa-related genes, i.e. GSTP1, APC, and RASSF1, which have been shown to be a significant predictor for the presence of PCa in histopathologically cancer-negative biopsies through a cancer-associated field effect [50]. In several different studies, ConfirmMDx was shown to have a NPV of 90% for all prostate cancers, and an NPV of 96% for clinically significant prostate cancers [49,51]. Furthermore, a ConfirmMDx clinical utility field study found that, of 138 men who received a negative assay result, only 6 men underwent repeat biopsies [52].

**4Kscore:** The 4Kscore test measures free and total (tPSA), human kallikrein 2 (hK2), and intact PSA to differentiate clinically insignificant tumors from aggressive PCa while also considering age, DRE results, and prior biopsy status [53]. The test facilitates early detection of aggressive disease (Gleason ≥ 7) while decreasing unnecessary secondary testing [54]. Gupta et al. reported in 2010 that considering a 4Kscore along with DRE and age in the decision for repeat biopsy would decrease the second biopsy rate by 712 per 1,000 patients, while 53 cancers would have a missed or delayed diagnosis [55]. A prospective study of 1,012 patients assessing the utility of 4Kscore in detecting high-grade PCa determined that the test could lead to a possible reduction in biopsies of 30%, with delayed diagnosis in only 1.3% of Gleason ≥ 7 PCa cases using a probability cutoff of ≥ 6% [56]. In another prospective study 42.8% of men could avoid biopsy, using a 6% risk of high-grade cancer as a cutoff, with 89.5% high-grade cancers detected and 10.5% missed [57].

**Conclusion**

Each year, more than 1 million American men undergo an invasive prostate biopsy with a negative result, however approximately 25% of those men actually have PCa. The current standard of care for prostate biopsy results in major and minor complications and samples less than
1% of the prostate. This leaves men at risk for undetected cancer and leads to a high rate of repeat biopsies, even on cancer-free men. Given the need for timely, actionable tools that can aid in the reduction of unnecessary repeat biopsies, this manuscript provides an overview of the current state of affairs in the repeat prostate biopsy setting and highlights available tools being used to help urologists ‘rule-out’ otherwise cancer-free men from undergoing unnecessary repeat biopsies and ‘rule-in’ men with high-risk disease.

It is well established that the utility of PSA as a diagnostic biomarker for prostate cancer is limited by the fact that only about 3% of PSA-screened men with PCa have lethal disease, thus leading to overtreatment of indolent disease [58]. Development of biomarkers to rule out lethal PCa at the point of screening address a great unmet clinical need, as this may reduce unnecessary interventions that may cause more harm than good. As biomarkers continue to evolve, they hold promise for future prostate cancer diagnosis and detection of clinically significant prostate cancer [59].

Other biomarkers that may be considered in the repeat biopsy space include SelectMDx (a reverse transcription PCR assay that may be considered in both the biopsy and repeat biopsy setting) [60] and Prostarix (a urine test that is based on a panel of biomarkers) [61]. Imaging is another option to aid in the detection of clinically significant PCa in the repeat biopsy setting-multi-parametric magnetic resonance imaging has been shown to detect significant PCa in men with prior negative biopsies [62]. The future will likely focus on further development and validation of promising biomarkers, exploring biomarker combinations (some research efforts have already demonstrated that combining biomarker panels may be a promising option for PCa diagnosis [63]) and prospective comparisons between markers.

Acknowledgment

The authors thank Karen Ventii, PhD, for editorial support.

References
