



The role of the liquid biopsy as a clinical tool for early prediction in prostate cancer

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Comment on: Van Neste L, Hendriks RJ, Dijkstra S, *et al.* Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker-Based Risk Score. *Eur Urol* 2016. [Epub ahead of print].

Submitted Aug 16, 2016. Accepted for publication Aug 19, 2016.

doi: 10.21037/tcr.2016.09.37

View this article at: <http://dx.doi.org/10.21037/tcr.2016.09.37>

The evolution of the liquid biopsy from a novel biomarker discovery platform to a clinical (molecular diagnostic) assay represents a true inflection point in the practice of medical (oncologic) pathology. Initially associated with the quantitation of breast cancer circulating tumor cells (CTCs) found in blood as a measure of tumor burden, the field quickly has expanded to include the isolation and capture of cell free/tumor DNA, exosomal RNA species and peptide-protein analytes in all body fluids including CSF and urine (1-4). The improvements in specimen handling, isolation techniques and the robust identification of low abundance nucleic acids have continued to advance the field; however, challenges persist as investigators attempt to understand the importance of rare variants in a complex setting of tumoral heterogeneity, drug resistance pathways and host-immune response. Recent success including the development of the first Food and Drug Administration (FDA) approved blood-based (liquid biopsy) companion diagnostic for the drug Tarceva (erlotinib) in patients with non-small cell lung cancer have further realized the potential (5). A simple, non-invasive, liquid biopsy approach for men with a suspicion of prostate cancer that offers insight into early detection of clinically significant disease while not over-diagnosing low-risk prostate cancer would have a critical impact on reducing the number of prostate needle biopsies and most importantly limiting over-treatment (6,7).

The current study by Van Neste *et al.* 2016 in the journal *European Urology* is an example of such a liquid biopsy assay, which relies on the isolation of cellular components found in post-digital rectal exam (DRE) total urine samples from men presenting to a urologist for either an initial or repeat biopsy. The primary objective of this study was to validate

the performance of a previously reported gene signature when combined with clinical variables would accurately predict high-grade (Gleason score 7) prostate cancer from GS6 and benign disease on prostate biopsy. There are currently two post-DRE urine assays commercially available including the United States FDA approved *PCA3* test (ProgenSA; Hologic) which detects *PCA3* mRNA transcripts normalized with *KLK3* (PSA) mRNA from sloughed epithelial cells and a second urine test that combines total serum PSA, the *PCA3* assay described above and the *TMPRSS2:ERG* fusion transcript known as the Mi-Prostate Score (MiPS) from the University of Michigan (8,9). The ProgenSA (*PCA3*) assay was originally FDA approved for men who had a prior negative biopsy but has shown efficacy in both the initial and repeat biopsy setting while the MiPS is currently used for both types of patients. These three assays require an 'attentive' DRE before urine collection and expedited specimen handling in a special transport tube and are able to predict a patient's risk for having both any prostate cancer and intermediate/high-grade GS7 disease. Furthermore, both the MiPS and the current Van Neste assay incorporate clinical variables directly into the test results to achieve optimal predictive accuracy.

Of note, a urine-based exosome-derived gene expression (*mRNA*) test which includes *PCA3* combined with total *ERG* (V-ets erythroblastosis virus E26 oncogene homologs) normalized with SAM pointed domain-containing Ets transcription factor (SPDEF) was recently validated to predict GS7 disease at initial biopsy for men with equivocal PSA from 2–10 ng/mL (10,11). In distinct contrast to the previously described urine tests, this assay does not require a DRE, and there is no need for expedited transport or

special handling. As with any new assay, in addition to accuracy, the ability to easily introduce into clinical practice will be a significant factor towards adoption. Furthermore, the exosome assay assesses total *ERG*, which also includes the fusion transcript addressing some of the recent reports that total *ERG* RNA levels are associated with clinical characteristics of higher risk prostate cancer (12,13).

Van Neste *et al.* used training and test cohorts (n=519 and n=386, respectively) which included men scheduled for either an initial or repeat biopsy based on an elevated PSA ≥ 3 , abnormal DRE, or family history of prostate cancer. All urine samples were collected after a standard DRE, subsequently transferred to a specialized carrier tube, shipped at room temperature and then stored at -80°C prior to analysis. Some important clinical characteristics are noted in the training and test cohorts, including fairly high median PSA values (16 *vs.* 12 ng/mL), high percentage of men with abnormal DRE's (38% *vs.* 31%) and a high percentage of $\geq$ GS7 prostate cancer (51% *vs.* 50%). Also noteworthy is the prior biopsy rate of 21 *vs.* 11%. In addition, the total combined cohort was predominantly (>95%) white. A prototype amplification kit was utilized for RNA isolation with a one-step RT-qPCR and the *KLK3* PSA gene used as a normalizer. Standard statistical analyses were employed including AUC of the ROC.

The authors in the current study compared a series of novel genes initially using a fixed sensitivity of 90% with pre-determined cut-offs and identified that the *homeobox C6* (*HOXC6*), and *distal-less homeobox 1* (*DLX1*) had the best combined AUC of 0.76 for predicting high grade disease. The gene combination was subsequently validated with an AUC of 0.73. They then introduced a series of clinical variables into the primary gene expression model to assess performance. Two models were created, $\pm$ DRE as an additional clinical risk factor that included both *HOXC6*, *DLX1*, combined with the clinical variables: PSAD, previous negative biopsy, total serum PSA, family history and age. With or without DRE risk factors, the AUC in validation ranged from 0.86–0.90. There are a few additional points worth noting. The authors observed that a model developed with only traditional clinical risk factors in the test cohort produced an AUC of 0.87 (by report mainly driven by PSAD) and that the addition of the two genes would increase the AUC to 0.90. Although the difference is statistically significant ($P=0.018$) it is not certain whether this will be clinically relevant.

In addition, when the final test model which included the gene signature was applied to men with a total serum PSA <10 ng/mL, the true 'gray zone' population where a biopsy decision is most challenging, the models AUC with or without DRE risk factors ranged from 0.78 to 0.86, respectively. Noteworthy is that the 'gray zone' population was limited to

264 men from the test cohort of which 86% had no or low grade G6 prostate cancer. The number of men who had a prior negative biopsy in this group was also not reported. The Prostate Cancer Prevention Trial risk calculator (PCPTRc) 2.0 (which includes percent free-PSA) was used as the main benchmark for all models performance (14). In the validation/test cohort, the PCPTRc v2.0 yielded an AUC 0.77 and when *PCA3* was included, the AUC increased to 0.80; however, in the gray zone population the PCPTRc AUC was 0.66 and with *PCA3* increased to 0.72.

It is widely accepted that integration of composite tools to define patient risk are important elements of personalized medicine. The more quantitative the outcome, the more precise and useful they become. Given the hazards of a prostate biopsy including infection, cost and diagnosis of low-risk, indolent prostate cancer, it is imperative that the clinician be well informed on the specifics surrounding the development of new assays prior to incorporation. This includes parameters of trial design, target population, accuracy metrics and ability to implement in clinical practice.

The current study was not designed to evaluate the PSA 2–10 ng/mL gray zone population presenting for their initial biopsy and although sub-group performance was quite good, the evaluable patient cohort is small and additional features including prior negative biopsy status would be helpful to understand performance. Additionally, as prostate cancer risk models move towards the prediction of clinically significant disease, it will become increasingly important to discriminate GS7 prostate cancer based on the International Society of Urological Pathology (ISUP) categorization of 3+4 *vs.* 4+3 as improved classifiers for evaluating significant disease (15). As part of this effort, investigators will need to provide false negative assessment of the clinical significant Gleason 4+3 population.

As demonstrated in the published literature, the performance of the urine-based gene expression only models to predict high-grade prostate cancer, including the Van Neste, are all quite comparable with AUC's that range from 0.68–0.73. Given the impact of clinical variables alone on performance, especially as observed in the current study, one possibility is to retain gene risk models as independent patient-specific phenotypes and have the treating physician use this information in conjunction with on-line clinical nomograms such as the PCPTRc 2.0 to facilitate more informed decision-making. Furthermore, an additional challenge is the requirement of a DRE prior to urine collection and the need for special specimen handling. These aspects may negatively affect general implementation in a busy clinical practice setting.

In closing, liquid biopsy assays, especially those derived from urine and blood, will no doubt advance and become fully

integrated into the precise 'diagnostic-prognostic' pathology tool kit. The appropriate assessment of these tests will continue to require diligence, along with extended validation and clinical utility studies to expand our understanding of their performance in sub-groups and ultimate impact on health outcomes. For patients and their treating physician, the ability to utilize a waste product to predict pathologic outcomes is an important milestone for the early detection (and future management) in the field of prostate cancer.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Peng Zhang (Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China).

Conflicts of Interest: The author is a clinical consultant to Exosome Diagnostics, Inc.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Donovan MJ. The role of the liquid biopsy as a clinical tool for early prediction in prostate cancer. *Transl Cancer Res* 2016;5(S3):S584-S586. doi: 10.21037/tcr.2016.09.37