Editorial

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The way of prostate cancer diagnostics

Editorial on "Comparative analysis of prostate cancer specific biomarkers *PCA3* and *ERG* in whole urine, urinary sediment and exosomes" by Hendriks RJ, Dijkstra S, Jannink SA, Steffens MG, Van Oort IM, Mulders PFA and Schalken JA

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Prostate cancer diagnostics has been essentially changed within the last 5 years. While prostate-specific antigen (PSA) remains the basic parameter, the additional value of the two 2012 FDA-approved biomarkers prostate health index (PHI) in serum and prostate cancer gene 3 (*PCA3*) in urine has been confirmed numerous times [1]. The detection of *TMPRSS2-ERG* gene fusions in the tissue of approximately 50% of all prostate cancer patients and the subsequently developed urinary assay [2] put hope on further diagnostic improvement that could unfortunately only be partially fulfilled [3–5].

The senior author of this article [6] in this issue of *Clinical Chemistry and Laboratory Medicine* played the key role in detecting *PCA3* and developing urinary assays for *PCA3* and *TMPRSS2-ERG* [7]. And this group is now the first that compared both markers in whole urine, urinary sediment and exosomes. With this new independent study, the authors completed and partly relativized previous data when they compared only the profile of these markers in urinary sediments and exosomes [8]. This comparative approach between various urine fractions can be considered as exemplary for testing other biomarkers not only for prostate cancer but also for renal cell carcinoma and bladder cancer. The positive effect of a digital rectal examination (DRE) of the prostate before urine sampling for diagnostic purpose was confirmed regarding the diagnostic validity of these markers in detecting prostate cancer. However, the main result was that whole urine results in a higher analytical sensitivity compared to sensitivity obtained using sediments and exosomes. In this respect, the advantage to use whole urine samples as applied in the tests for *PCA3* and *TMPRSS2-ERG* instead sediments is not only justified from the practical point of view but also with regard to the improved analytical sensitivity. On the other hand, biomarker levels measured in the three tested urine fractions in this study and presented in table 4 proved that there was a distinct difference between the levels in the whole urine and the sum of the two other fractions [6]. Thus, it can be concluded that a great amount of these mRNAs in the urine obviously occurs in free forms without any association to particles (exosomes) and without cellular confinement (sediments). It is particularly worth mentioning that this comprehensively researched study by Hendriks et al. clears away the erroneous view that nucleic acids in urine as in this case of *PCA3* mRNA and *TMPRSS2-ERG* mRNA are mostly detected in the released prostate cells. In consequence, the analytical focus on sediments as done in several studies does not let expect satisfying results. A similar phenomenon of differences between samples of whole urine, cell-depleted urine, and sediments was also observed in bladder cancer patients for various mRNAs [9]. In addition, the differences in that study were not uniform for all tested mRNAs but showed a particular behavior for specific mRNAs [9]. Thus, the focus on possible new urinary markers including non-coding nucleic acids like microRNAs, long non-coding RNAs, and piRNAs should draw attention to this aspect. These observations imply that whole urine analysis as starting point should be used before the markers are tested regarding their diagnostic usefulness in the different urine fractions. Even more, each new marker can be reliably assesed if measured in all urinary fractions.

Urine as a complex substrate with several fractions is not easy to handle and processing and mRNA or miRNA extraction depends on many factors including stability. Regarding sample stability and storage, whole urine further can be surely preferred since different commercially available procedures have been recommended. These technical devices, e.g. supplied by Norgen Biotek Corp., Thorold, Canada with its various kits for urine RNA concentration, preservation, and isolation facilitate the applicability of these nucleic acid-based markers in urine

in practice despite reliable long-term storage data are missing so far.

However, from the analytical aspect it is easier to handle serum. (-2)proPSA and the formula (-2)proPSA/freePSA × √PSA, which is named prostate health index (PHI) shows a better correlation with tumor aggressiveness than PCA3 [1]. Despite a clear clinical usefulness of *PCA3* [10] its limitations are the relative complicated measurement procedure and the low sensitivity at high values of >100 [11]. However, the combination of *PCA3* and *TMPRSS2-ERG* scores within several PSA-based models improved the predicting of PCa and high-grade PCa [5]. But it should be noted that the *PCA3* and *TMPRSS2-ERG*-based Michigan-Prostate Score (MiPS) has costs of ~750 $. While this test improves prostate biopsy indication it cannot replace the biopsy itself. Here a multiparametric magnetic resonance imaging (MRI) and in cases of suspicious lesions by using the Prostate Imaging Reporting and Data System (PI-RADS) a subsequent MRI/ultrasound fusion biopsy is done [12]. A clinical aggressive and significant PCa will be almost always detected by MRI with a detection rate of up to 87% in summarized data of >1900 patients [12]. However, in case of a non-suspicious PI-RADS score ~27% of mostly Gleason 3+3=6 cancers are overlooked as they are exclusively detected in the additional systematic biopsies after MRI/ultrasound fusion biopsy [13]. However, despite this shift in PCa diagnostics towards PI-RADS score based MRI and MRI/ultrasound fusion biopsies in patients with or even without prior biopsy we propose a significant role for biomarkers in serum and urine. For example, a young man with a gray zone PSA of 2–10 µg/L and non-suspicious MRI may still suffer from a Gleason 3+3=6 PCa and the presence of high values of PHI, PCA3 or MiPS may help to force a subsequent systematic biopsy. Thus, the time of prostate biomarkers is not over but it should be used in combination with the MRI in an appropriate strategy. So far, there is only one study that compared MRI, PHI and PCA3 with an advantage for the MRI but no PIRADS score was used [14]. Further comparisons of the established and new biomarkers with the MRI are necessary to find the best possible PCa diagnostic pathway of the future.

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