Urine as a liquid biopsy - is it the holy grail?

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INTRODUCTION

The predicted global cancer burden is expected to increase significantly – By 2040, 29.5 million new cancer cases are estimated, compared to 18.1 million cases reported worldwide in 2018[1].

Early detection of cancer can greatly increase chances of survival and improve overall quality of life of a patient. Cancer biomarkers* can be found in tissues as well as bodily fluids and can be used to detect the disease early as well as monitor disease progression[2].

An ideal biomarker must offer high sensitivity and high specificity, meaning it should only be detectable in case of disease. Additionally, biomarker levels can help understand the severity of disease through predictive and prognostic values[3].

A high number of potentially informative cancer biomarkers have been found, including DNA, RNA, proteins and metabolites. This has been made possible through the ability to sequence the entire human genome as well as advances in key technologies such as high throughput DNA sequencing, microarrays, and mass spectrometry[2].

WHEN TISSUE IS AN ISSUE

Biopsies are essential to achieve the objectives of precision oncology and allow for targeted therapies based on the genetic profile of the disease. A tissue biopsy is the traditional approach used to diagnose many cancers. However, obtaining a tissue sample is not always feasible and the process can be invasive, painful, expensive, time-intensive, difficult and requires the intervention of a clinician[4-6]. In addition, due to intratumor heterogeneity, in some instances, the entire tumor landscape may not be reflected by a tissue biopsy.

As a result, researchers are continuously exploring alternative methods to detect cancer types. The use of minimally invasive procedures such as liquid biopsies and detection of circulating tumor markers in body fluids is gaining interest[6].

Circulating molecules such as cell-free DNA, circulating tumor cells, circulating RNAs, proteins, peptides and exosomes can provide a global view of primary and metastatic tumors. Circulating molecules can be detected in various biological fluids, including cerebrospinal fluid, plasma, saliva, seminal plasma, serum and urine.

Liquid biopsies have several advantages - they allow (repeated) sampling, providing a personalized snapshot of a disease at successive time points. Additionally, they can offer a solution to tumor heterogeneity, and better reflect the genetic profile of all tumor subclones, as opposed to tissue biopsies which are obtained from one tumor region. Liquid biopsies are also associated with significantly less morbidity and can prevent complications associated with traditional biopsies[6].

YELLOW IS THE NEW RED

The most often used liquid biopsy is blood, which uses either serum or plasma as a sample type. However, blood as a liquid biopsy has several limitations that have hampered its development as clinically useful biomarker test. Blood has the drawback that it has a relatively high and complex protein repertoire. Further, components of the blood matrix can also interfere with biomarker measurements. The invasive nature of blood tests also limits access to repeated measurements and poses a risk of infection for both the patient and caregivers, along with the additional costs of minimizing this risk[7].

Urine has been proposed as an alternative biofluid for detecting and monitoring treatment of urological and systemic cancers. Urine is easily accessible, non-invasive, available in larger quantities and applicable for home collection[7]. Moreover, the collection of urine is not limited by the health status of a patient[8] and does not entail any risk of transmission of blood-borne pathogens[9]. In addition, urine testing enables cost-efficient rapid and serial sampling, allowing for patient monitoring as well as reproducibility of assays[7,8]. In terms of analysis, the isolation of DNA from urine is in theory easier than blood, due to the low protein content after filtration in the kidney[7,10].

Several studies have shown that the use of urine as a liquid biopsy for cancer detection and monitoring is promising due to the ease of sampling and high acceptability compared to blood and tissue[6,11-13]. Urine cell free tumor DNA has proven to be of value in biomarker studies of bladder, kidney and prostate cancer, but surprisingly also in breast, colon and lung cancer[12,14-16].

COLLI-PEE® AND RESEARCH

To use urine for clinical applications, the preanalytical variation (collection, transport and storage) must be kept to a minimum. A standard urine cup has limitations and can be awkward, messy and inconvenient for the user. These can be overcome with Novosanis' device, Colli-Pee®, which is a collection device that allows for standardized and volumetric collection of urine and ensures immediate mixing with preservative[11,17]. Colli-Pee® is a user-friendly method to capture first-void/first-catch urine (first volume of urine flow), improving sample collection for downstream analysis. First-void urine contains a higher concentration of DNA than other fractions of urine, improving diagnostic sensitivity [17,18].

Several studies using Colli-Pee® have focused on detection of Human Papillomavirus (HPV), the leading cause of cervical cancer[11,19-23]. Some of the publications and results are part of large clinical studies including EVAH, Predictors5.1 and VALHUDES[19,22,24].

Figure 1: Novosanis' Colli-Pee® 20 mL variant

CONCLUSION

Urine as a liquid biopsy offers a huge potential for cancer biomarker testing. Several diagnostic assays using urine are commercially available or on the verge of launch in the clinical practice. We believe urine can be considered the holy grail in cancer biomarker testing, enabling cancer detection and follow-up in a non-invasive and easy way.

*The World Health Organization (WHO) defines a biomarker as "any substance, structure or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease"

References:

(**1)** Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

(2) Gupta AK, Reddy VP, Lavania M, et al. jefA (Rv2459), a drug efflux gene in Mycobacterium tuberculosis confers resistance to isoniazid & ethambutol. Indian J Med Res. 2010;132:176-188.

(3) Badila E, Japie C, Bartos D. Cancer biomarkers in clinical practice. Rom J Intern Med. 2014;52(4):223-232.

(4) Paudyal P, Llewellyn C, Lau J, Mahmud M, Smith H. Obtaining self-samples to diagnose curable sexually transmitted infections: a systematic review of patients' experiences. PLoS One. 2015;10(4):e0124310.

(5) Shih SL, Graseck AS, Secura GM, Peipert JF. Screening for sexually transmitted infections at home or in the clinic? Curr Opin Infect Dis. 2011;24(1):78-84.

(6) Di Meo A, Bartlett J, Cheng Y, Pasic MD, Yousef GM. Liquid biopsy: a step forward towards precision medicine in urologic malignancies. Mol Cancer. 2017;16(1):80.

(7) Nolen BM, Lokshin AE. -The advancement of biomarker-based diagnostic tools for ovarian, breast, and pancreatic cancer through the use of urine as an analytical biofluid. Int J Biol Markers. 2011;26(3):141-152.

(8) Franovic A, Raymond VM, Erlander MG, Reckamp KL. Urine test for EGFR analysis in patients with non-small cell lung cancer. J Thorac Dis. 2017;9(Suppl 13):S1323-S1331.

(9) Dudeck MA, Edwards JR, Allen-Bridson K, et al. National Healthcare Safety Network report, data summary for 2013, Device-associated Module. Am J Infect Control. 2015;43(3):206-221.

(10) Su YH, Wang M, Brenner DE, et al. Human urine contains small, 150 to 250 nucleotide-sized, soluble DNA derived from the circulation and may be useful in the detection of colorectal cancer. J Mol Diagn. 2004;6(2):101-107.

(11) Pattyn J, Van Keer S, Biesmans S, et al. Human papillomavirus detection in urine: Effect of a first-void urine collection device and timing of collection. J Virol Methods. 2019;264:23-30.

(12) Liu Z, Liu W. Association of urinary and plasma DNA in early breast cancer patients and its links to disease relapse. Clin Transl Oncol. 2018;20(8):1053-1060.

(13) Xiao D, Zeng Y, Singh SV. Diallyl trisulfide-induced apoptosis in human cancer cells is linked to checkpoint kinase 1-mediated mitotic arrest. Mol Carcinog. 2009;48(11):1018-1029.

(14) Fujita K, Nonomura N. Urinary biomarkers of prostate cancer. Int J Urol. 2018;25(9):770-779.

(15) Krishnamurthy N, Spencer E, Torkamani A, Nicholson L. Liquid Biopsies for Cancer: Coming to a Patient near You. J Clin Med. 2017;6(1).

(16) Theodorescu D, Schiffer E, Bauer HW, et al. Discovery and validation of urinary biomarkers for prostate cancer. Proteomics Clin Appl. 2008;2(4):556-570.

(17) Vorsters A, Van den Bergh J, Micalessi I, et al. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis. 2014;33(11):2005-2014.

(**18)** Johnson DJ, Calderaro AC, Roberts KA. Variation in nuclear DNA concentrations during urination. J Forensic Sci. 2007;52(1):110-113.

(19) Leeman A, Del Pino M, Molijn A, et al. HPV testing in first-void urine provides sensitivity for CIN2+ detection comparable with a smear taken by a clinician or a brush-based self-sample: cross-sectional data from a triage population. BJOG. 2017;124(9):1356-1363.

(20) Franceschi S, Chantal Umulisa M, Tshomo U, et al. Urine testing to monitor the impact of HPV vaccination in Bhutan and Rwanda. Int J Cancer. 2016;139(3):518-526.

(21) Vorsters A, Van Keer S, Biesmans S, et al. Long-Term Follow-up of HPV Infection Using Urine and Cervical Quantitative HPV DNA Testing. Int J Mol Sci. 2016;17(5).

(22) Arbyn M, Peeters E, Benoy I, et al. VALHUDES: A protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples. J Clin Virol. 2018;107:52-56.

(23) Van Keer S, Tjalma WAA, Pattyn J, et al. Human papillomavirus genotype and viral load agreement between paired first-void urine and clinician-collected cervical samples. Eur J Clin Microbiol Infect Dis. 2018;37(5):859-869.

(24) Cuzick et al, ASCCP 2019