Editorial

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Liquid profiling – circulating nucleic acid diagnostics gains momentum

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Liquid profiling of molecular characteristics of circulating nucleic acids (CNAs) in the blood and other bodily fluids is one of the most interesting, dynamic and promising areas in laboratory diagnostics. Since the last special issue on the diagnostics of cell-free nucleic acids (J Lab Med 2016), the field has expanded rapidly in various directions and is about to reach routine clinical application in non-invasive prenatal testing, oncology and transplantation medicine.

With the optimization of new technologies such as massive parallel sequencing, standardization and rigorous quality control, disease-specific enrichment of nucleic acids, combination of diverse molecular characteristics and advanced bioinformatic pipelines, the diagnostic power of liquid profiling has been considerably increased. It also has become evident that a better understanding of the biology, structure, function and metabolism of CNAs is crucial for improving the diagnostic process and for accurate interpretation of the results.

In a recent conference on "Circulating Nucleic Acids in Plasma and Serum (CNAPS)" held at San Francisco in May 2022, many aspects were addressed by pioneers and world-leading specialists in the field, including (i) new developments in the application transplant and infectious disease diagnostics, (ii) early cancer diagnostics and monitoring of cancer disease, (iii) maternal and fetal diagnostics, (iv) utilization of new approaches in epigenetics, fragmentomics and tissue deconvolution, and (v) discussion of future perspectives and emerging frontiers. While many studies provided strong clinical evidence for the diagnostic utility of CNAs, the meeting clearly revealed the different pace at which CNA diagnostics is implemented in the diverse national health care systems [1].

The collection of articles in this new Special Issue by the J Lab Med offers an update on some major topics of nucleic acid diagnostics: Bronkhorst et al. give a broad overview on the diverse forms of nucleic acids found in bodily fluids, their sources, release and metabolism, pre-analytical and analytical improvements that enable sensitive and quantitative assessment, as well as next steps from snapshot diagnostics towards individualized monitoring of temporal genomic changes [2].

The long and arduous route that liquid profiling had to take until being accepted for patient care is outlined by Haselmann et al. The review provides a succinct depiction of the current status of CNA testing for patient stratification, detection of resistance mechanisms and treatment monitoring in cancer patients, and also highlights the challenges toward successful clinical implementation [3].

Froelich et al. report an example of how the acceptance of liquid profiling can be increased, and how its integration into clinical workflows of routine patient care be boosted. By delivering relevant information for an appropriate selection of targeted therapies, liquid profiling can become an integral part of local molecular tumor boards (MTBs) supplementing tissue diagnostics and enabling "true" companion diagnostics with personalized molecular markers during the course of cancer disease. In an interdisciplinary approach, liquid profiling can be utilized in combination with highly sensitive imaging, thereby triggering and complementing each other in a stepwise manner, in order to overcome the intrinsic limitations of both methods and to leverage their full potential for personalized therapy guidance [4].

As the prognosis of cancer is more favourable when the patient is treated in an early, locally confined stage and since cancer-related changes can be detected in the blood plasma, highly sensitive technologies for the detection of circulating tumor DNA (ctDNA) have become a possibly powerful diagnostic tool for early cancer detection and object of intensive research. In contrast to earlier attempts

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with protein tumor markers, novel approaches include multiple molecular features and address multiple tumor types at the same time. Holdenrieder et al. summarize the current status of the so-called pan-cancer screening by ctDNA and outline the limitations and pitfalls but also the perspectives for ctDNA-based screening in the future [5].

It has become evident that the complex pathology of cancer disease cannot be completely captured by one biomarker, a single class of biomarkers or a single analytical compartment. Keup et al. show in their review and their impressive study on the use of multi-modal and multi-analyte liquid biopsy testing in metastatic breast cancer patients (ELIMA) how the combined analysis of DNA and RNA from blood plasma, circulating tumor cells (CTCs), and extracellular vesicles (EVs) – all done from one single blood draw – may address different aspects of cancer disease, but at the same time provide complementary information [6].

Beyond deriving from different sources, the biological behaviour and function of nucleic acids in the blood is also of interest for the diagnostic process itself. Ricklefs et al. depict the various forms of cell-free DNA (cfDNA) circulating solely in plasma, being associated with EVs, nucleosomes or other proteins or being part of so-called neutrophil extracellular traps (NETs) and their possible role in tumor progression and estimation of prognosis [7].

It is self-evident that, for reproducible and clinically meaningful diagnostics of novel biomarkers like extracellular vesicles (EVs), all processes must be standardized and highly quality-controlled. Enderle and Noerholm summarize the necessary steps that must be taken for proper sample collection, robust preanalytics, most effective sample extraction and choice of appropriate EV detection method. Being a great platform for future diagnostics, the greatest challenge of EVs is currently the identification of robust and clinically meaningful biomarkers [8].

While liquid profiling of tumor-specific molecular changes of ctDNA has improved cancer patient characterization, it requires the highly sensitive detection by optimized next generation sequencing (NGS) technologies. But beyond looking at the "needle in the haystack", the haystack itself is of great interest for profiling disease and tissue-specific diagnostics. Oberhofer et al. give an overview on current procedures for utilizing information from epigenetic signatures and DNA fragmentation patterns for exploring the source of cfDNA and the underlying pathology [9].

Importantly, cfDNA is also released under physiological conditions such as physical activity during endurance or strength training, and as reported by Neuberger et al. cfDNA derives mainly from neutrophils and is liberated within minutes during a process called "vital NETosis". On the one hand this feature may be useful for evaluating training effects, but it also has to be considered as an influencing factor because rare DNA fragments like ctDNA or fetal DNA are diluted and make sensitive diagnostics more difficult [10].

Another issue that has both beneficial and unfavourable impacts on cfDNA diagnostics is clonal hematopoiesis of indeterminate potential (CHIP). This comprises somatic mutations in peripheral blood cells without evidence for the presence of solid or liquid neoplasms. As outlined by Gregor Hoermann CHIP associated mutation profiles represent a kind of biological noise in liquid profiling diagnostics, but they also have their own clinical relevance as risk factors for hematologic malignancies as well as non-hematological pathologies like cardiovascular diseases that have to be explored further in the near-future [11].

In contrast to liquid profiling in cancer disease, non-invasive prenatal testing (NIPT) of circulating cell-free fetal DNA (cffDNA) in maternal blood plasma is already clinical routine and has recently achieved reimbursement from the statutory insurance. Thus, NIPT can serve as blueprint for all other types of liquid profiling testing. Kypri et al. present an update of NIPT 2022 and outline the different diagnostic approaches, achievements and ongoing challenges [12].

As one approach to push the implementation of liquid profiling of cancer-related molecular patterns into clinical patient care, the EXLIQUID initiative of the German Cancer Consortium has been established as an interdisciplinary working multicentric network that aims at exploiting liquid biopsies to advance cancer precision medicine for molecular tumor board patients. Mack et al. report on this novel academic German research structure, the aims and workplan as well as the projects of the many partner sites that include comprehensive molecular testing of plasma nucleic acids also to find new patterns in rare cancer types that can be addressed by targeted therapies [13].

The field of circulating nucleic acids is rapidly evolving and gaining momentum each year, and within the scope of this Special Issue we could only address some of the current topics. Beyond NIPT and cancer diagnostics, diagnostic approaches in transplantation medicine and infectious diseases will soon be available for the clinical routine. Furthermore, we will learn about new approaches for cfDNA characterization in the blood, combination of technologies, and the use of advanced bioinformatics and machine learning to manage and interpret the enormous datasets generated in the field [14, 15]. Finally, we still need more research on the biology, structure, function, release and metabolism of CNAs to better understand

their biological and clinical meaning [15, 16]. For the implementation into clinical practice, quality management aspects of liquid profiling will soon be addressed by the new D5 chapter of the Rili-Baek. But despite gaining momentum, liquid profiling will only be broadly accepted as the diagnostic "game changer" if reimbursement is further expanded.

We hope that you enjoy with us this Special Issue of the *Journal of Laboratory Medicine*, which covers a wide range of new developments on blood-based nucleic acid diagnostics, and we thank all the contributing authors.

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