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A global perspective on the status of clinical metabolomics in laboratory medicine – a survey by the IFCC metabolomics working group

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Abstract

Objectives: Metabolomics aims for comprehensive characterization and measurement of small molecule metabolites (<1700 Da) in complex biological matrices. This study sought to assess the current understanding and usage of metabolomics in laboratory medicine globally and evaluate the perception of its promise and future implementation.

Methods: A survey was conducted by the IFCC metabolomics working group that queried 400 professionals from 79 countries. Participants provided insights into their experience levels, knowledge, and usage of metabolomics approaches, along with detailing the applications and methodologies employed.

Results: Findings revealed a varying level of experience among respondents, with varying degrees of familiarity and utilization of metabolomics techniques. Targeted approaches

dominated the field, particularly liquid chromatography coupled to a triple quadrupole mass spectrometer, with untargeted methods also receiving significant usage. Applications spanned clinical research, epidemiological studies, clinical diagnostics, patient monitoring, and prognostics across various medical domains, including metabolic diseases, endocrinology, oncology, cardiometabolic risk, neurodegeneration and clinical toxicology.

Conclusions: Despite optimism for the future of clinical metabolomics, challenges such as technical complexity, standardization issues, and financial constraints remain significant hurdles. The study underscores the promising yet intricate landscape of metabolomics in clinical practice, emphasizing the need for continued efforts to overcome barriers and realize its full potential in patient care and precision medicine.

Keywords: clinical metabolomics; clinical lipidomics; emerging technologies; diagnostics; prognostics; laboratory medicine

Introduction

Metabolomics is a discipline in which one or several analytical techniques based on mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy are used to measure a large set of metabolites present in a biological sample. Highly conserved primary metabolites involved in energy metabolism (as fuels), maintenance of cell structure (as building blocks) and metabolic signaling are of particular interest in metabolomics due to the crucial roles they play in cellular function, maintenance, differentiation, growth and death. The high chemical diversity (volatility, polarity, etc.) of these metabolites as well as the wide concentration range spanning at least 11 orders of magnitude in biological samples [1] has led to the division of the discipline into several subcategories which include, for example, lipidomics, volatolomics, steroidomics and microbiome metabolomics. In addition to endogenous compounds, metabolomics is also a well-suited approach to measure xenobiotic compounds, which makes it an ideal technique for clinical toxicology.

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One of the greatest expected benefits of metabolomics is that it allows a holistic approach to patient health monitoring and thereby, a shift from the current “one size fits all” to patient-centric or individualized model of care [2, 3].

Two main methodological approaches can be distinguished:

- (1) Targeted metabolomics, involving analysis of a select set of chemically characterized and biochemically annotated metabolites implicated in a single or multiple pathways that in aggregate are associated with a particular pathology or clinical condition of interest.
- (2) Untargeted metabolomics, a data-driven and hypothesis-generating approach involving comprehensive profiling of as many metabolites as possible, primarily to identify novel biomarkers for subsequent targeted clinical use or disease-associated patterns that, after thorough replication and validation, might have (1) predictive value – for risk prediction, (2) diagnostic value – to identify the disease onset or development in its early stage and (3) prognostic value – to evaluate the rate of disease progression.

Clinical applications of metabolomics have been reported for disease biomarker discovery and monitoring [4], precision medicine [5] and the monitoring of treatment responses [6].

In 2021, the emerging technology division of the IFCC created a metabolomics working group with the following objectives:

- (1) Monitoring emerging trends in the field of metabolomics
- (2) Providing regular updates on the applications of metabolomics in clinical diagnosis and prognosis
- (3) Assessing the accessibility of, and barriers to, routine implementation of metabolomics approaches and methodologies in clinical laboratories

The IFCC working group on metabolomics designed a questionnaire to gain a comprehensive overview of the current use of metabolomics in clinical laboratories globally.

In this report, we summarize the findings of the survey (comprising 30 questions) that was conducted in October, 2023. Additionally, we provide some perspectives on the current state of metabolomics and issues impacting its broader clinical translation.

Methods

The questionnaire was conducted using the online survey tool SurveyMonkey (surveymonkey.com) from the 20th of October 2023 to the 5th of November 2023 and disseminated through the IFCC mailing list. In

addition, participation in the survey was encouraged through social media.

Results

General description of participants (Q1–Q4)

A total of 400 professionals participated in the survey, representing 79 countries. By region, respondents were from Europe (182), Asia (110), Africa (47), South America (41), North America (15) and Oceania (5) (Figure 1).

Thirty 5 % of respondents indicated they work at university hospitals, 15 % were employed by university or research institutes, and 14 % by privately owned laboratories (others: government (12 %), non-university hospital (10 %), private health care center (7 %), industry (4 %), other (4 %)).

Thirty percent were specialists in laboratory medicine, clinical biology or pathology, 29 % were lab manager/director/supervisor/coordinator, 9 % laboratory technician/medical technologist (others: 7.5 % scientist, 7 % student (graduate, PhD, resident), 6 % researcher, 2 % retired/emeritus, 1.5 % engineer, 1 % *In Vitro* Diagnostics industry, 8 % other).

Regarding the types of analysis performed by the respondents, 69 % indicated more than one field of activity. The breakdown was as follows: 76 % clinical biochemistry, 46 % immunology, 46 % cytology, 42 % coagulation, 34 % microbiology, 33 % molecular biology, 28 % point of care testing, 24 % inborn errors of metabolism, 21 % toxicology, 21 % therapeutic drug monitoring, 17 % transfusion medicine, 19 % other. Four percent indicated their institution does not perform any clinical analysis (Figure 2, Q2–Q4).

Participants' experience level, knowledge and use of metabolomics (Q5–Q7)

Overall, 59 % of respondents reported having at least 10 years of experience in their fields of expertise, 24 % had 5–10 years of experience, while 18 % had less than 5 years of experience. The majority (57 %) considered themselves to have very little or only a basic knowledge of metabolomics. Twenty-seven percent reported having good or very good knowledge, while only 8 % considered themselves expert in the field. One hundred and seventy three participants (50 %) declared that they had used or were currently using metabolomics assays, either in their own labs, with collaboration partners, or from a commercial service provider (Figure 2, Q5–Q7). Of the commercial solutions, Biocrates and

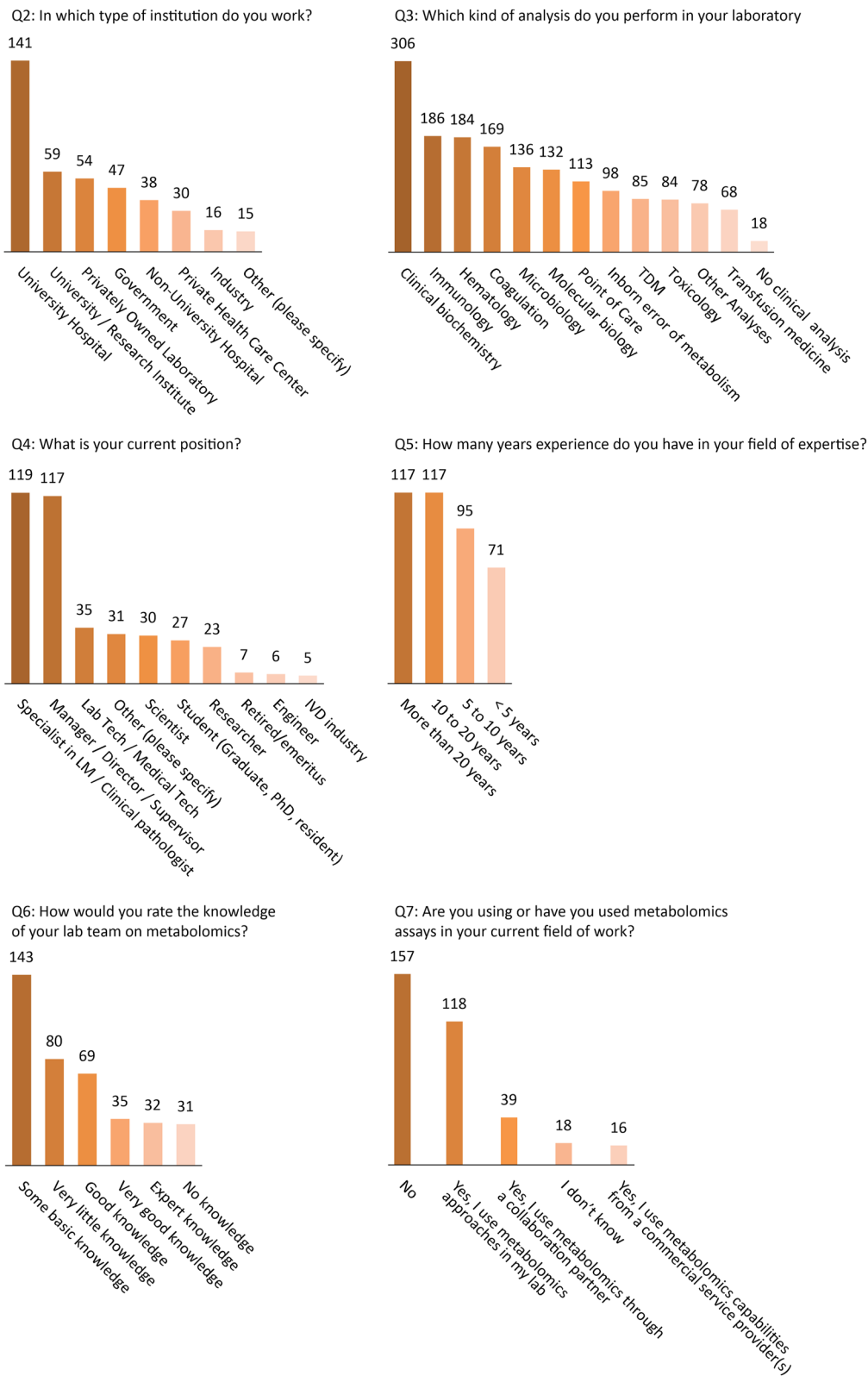
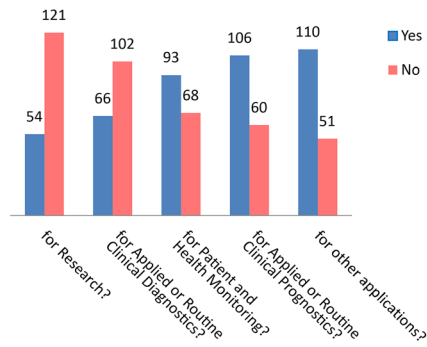
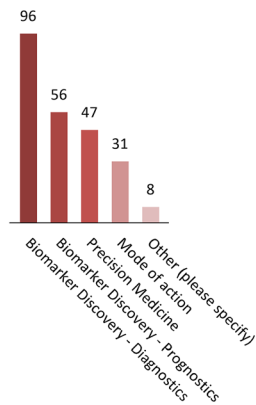


Figure 2: Basic characteristics of participants (Q2–Q4) and experience level of participants and their knowledge and use of metabolomics (Q5–Q7).

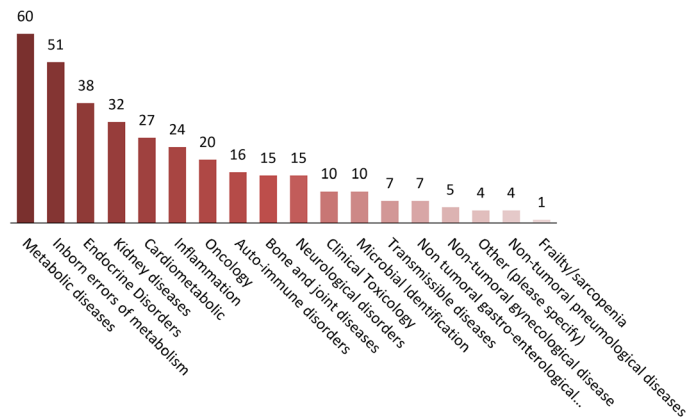
Q8-Q12: In which areas do you use clinical metabolomics?



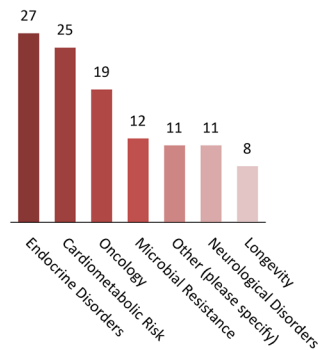
Q13: As you use Clinical Metabolomics for Research, please indicate in which field you use it.



Q14: As you use Clinical Metabolomics in the Applied or Routine Clinical Diagnostics, please indicate in which area/field you use it.



Q15: As you use Clinical Metabolomics in Applied or Routine Clinical Prognostics, please indicate in which area/field you use it



Q16: As you use Clinical Metabolomics in Patient or Health Monitoring, please indicate in which area/field you use it

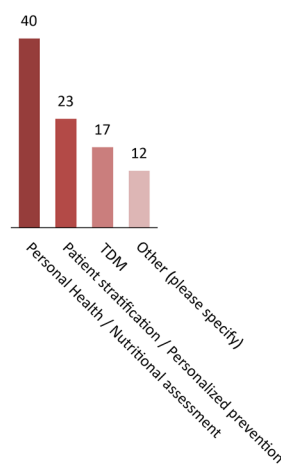


Figure 3: Applications of clinical metabolomics.

considered as main hurdles in the implementation of metabolomics assays.

The opinions about the medical value of metabolomics-based assays were less varied, with 45 % of participants considering that metabolomics assays hold clinical utility vs. 35 % who believe there is a lack of medical value, while 20 % did not disclose their opinion (Figure 5).

Discussion

This survey was conducted by the IFCC metabolomics working group to evaluate the current understanding and usage of metabolomics in laboratory medicine, and the perception of the community about the promise and future implementation of this technology.

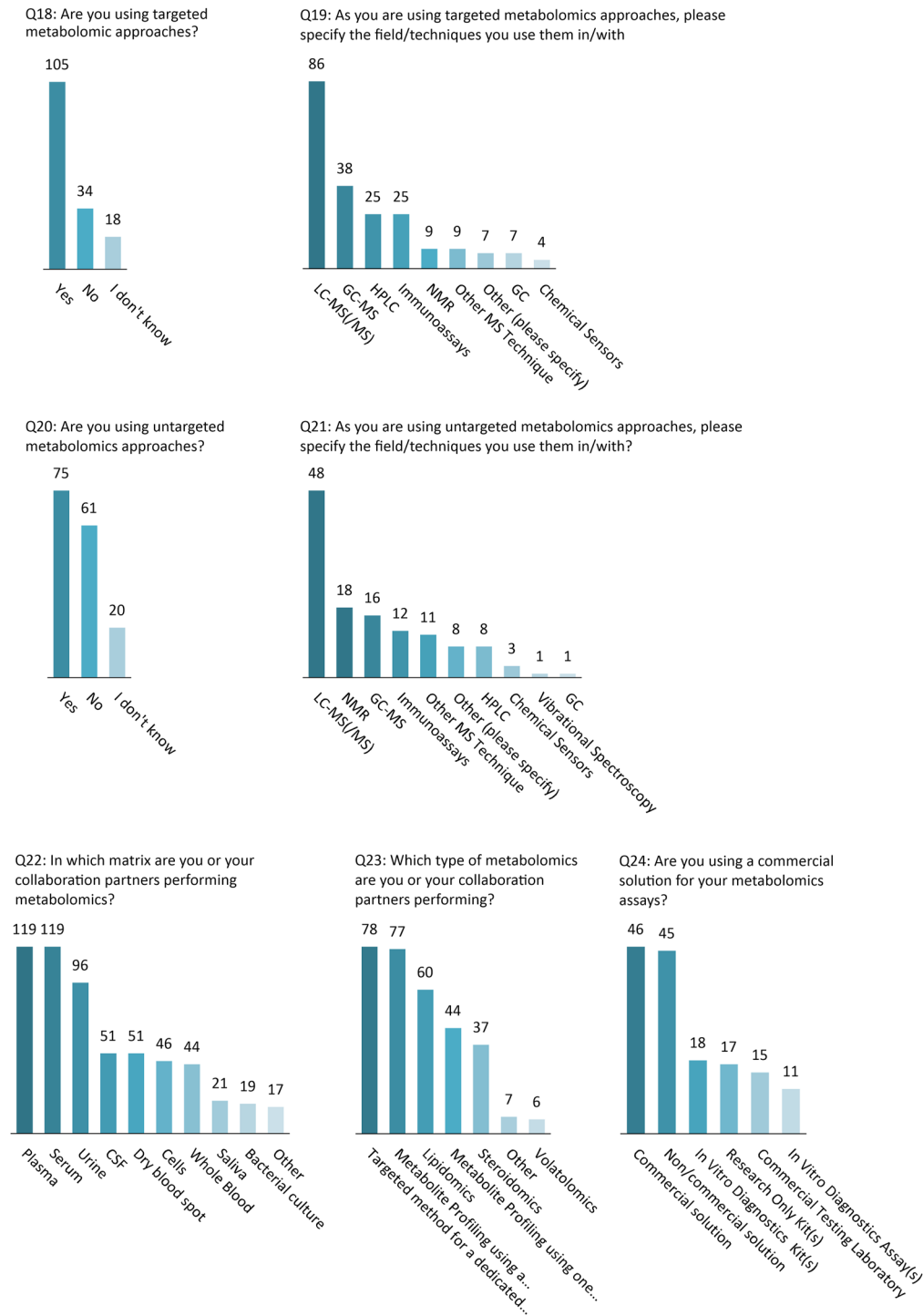
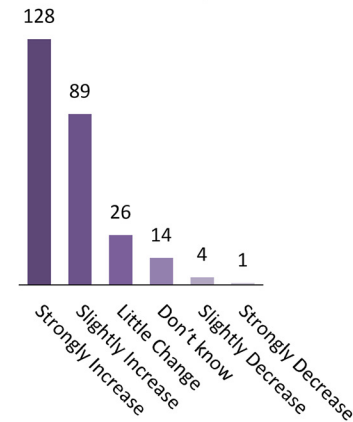


Figure 4: Technological approaches and methodologies used in clinical metabolomics.

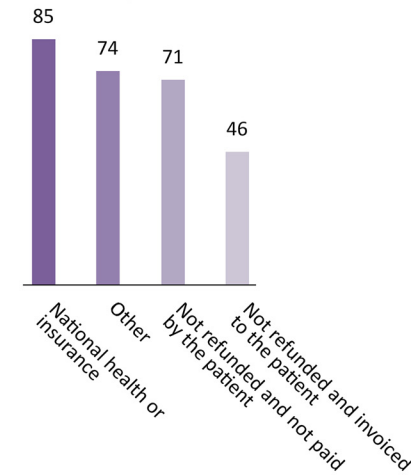
The relatively high participation rate (400 respondents) reflects the interest of the clinical community in metabolomics. The following definition of metabolomics was provided at the beginning of the survey: “Metabolomics is defined as the comprehensive characterization and simultaneous measurement of a large number of metabolites and

low-molecular-weight molecules in a biological specimen. Lipidomics, volatilomics and steroidomics belong to the field of metabolomics. Users of lipidomics or other -omics of small molecules can therefore answer the questions as metabolomics users.” A fairly large percentage of our respondents (72 %) reported having at least basic knowledge of

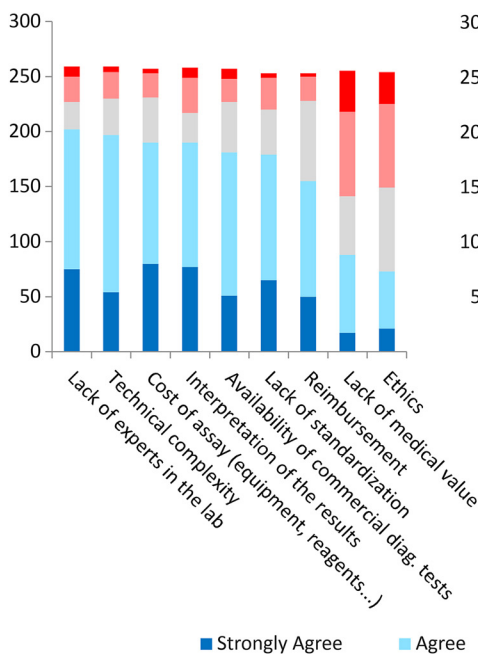
Q26: How do you think implementation of metabolomics for patient care will evolve in the next five years?



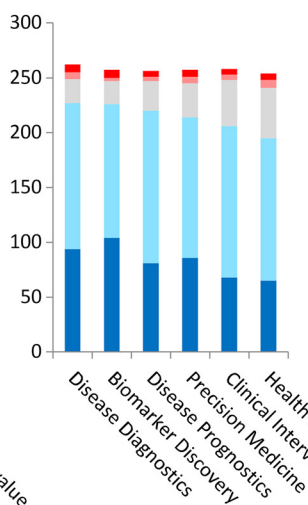
Q30: How are the metabolomics analyses you perform in the clinical setting refunded?



Q27: Please rate the following factors as being the main hurdles for the implementation of metabolomics in clinical labs.



Q28: Which areas of medicine will likely benefit from metabolomics advancements in the future?



Q29: Which specific areas of clinical chemistry / laboratory medicine will likely benefit from metabolomics advancements in the future?

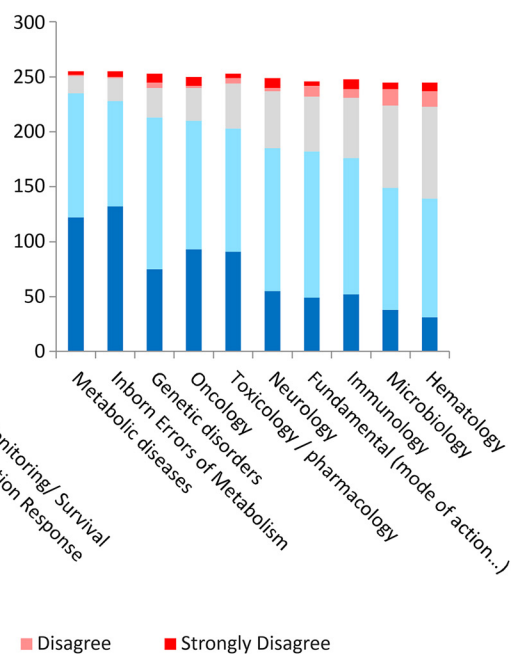


Figure 5: Future of clinical metabolomics (numbers next to the bars represent the number of responses).

metabolomics and half of them have already used metabolomics techniques. Somewhat misleading answers were received when the participants were questioned about the extent of their experience with metabolomics. More than 25 % (n=117) reported they had more than 20 years of experience, which is an unrealistic number likely reflecting the community's misunderstanding of the term metabolomics in general.

Approaches and methodology

There are two approaches to conduct metabolomics studies broadly defined as targeted and untargeted methods. Our survey indicates that in clinical metabolomics, targeted approaches partially dominate (n=105) vs. non-targeted approaches (n=75), while a significant number of participants are using both methods (n=59).

Targeted methods are used when specific pathways, biomarkers or classes of metabolites are of interest. The most prevalent technique used for targeted metabolomics was LC-MS (86 %), which offers significantly better sensitivity and selectivity compared to other techniques, especially when triple quadrupole MS are used in multiple reaction monitoring mode [7]. The use of GC-MS, immunoassays and HPLC techniques were also reported to a lesser extent for targeted methods.

Untargeted methods can be used for exploratory purposes to identify new biomarkers, changes in metabolic pathways or for screening the presence of xenobiotic compounds in clinical toxicology. LC-MS is also the technique that was the most commonly used for non-targeted approaches (64 %) followed by NMR (24 %) and GC-MS (21 %). Although LC-MS has the advantage of being more sensitive and of offering a larger metabolite coverage than the other techniques, it must be noted that NMR analysis has the unique benefits of being non-destructive, providing quantifiable results (also in untargeted analysis), requiring little or no separation [8]. GC-MS also has some distinct advantages over the two other techniques such as high sensitivity for volatile and semi-volatile low molecular weight compounds while providing a high chromatographic resolution, making it a powerful technique for volatolomics applications. Several participants reported the use of a combination of techniques LC-MS/GC-MS/NMR ($n=7$), LC-MS/NMR ($n=6$) and LC-MS/GC-MS ($n=6$) allowing them to substantially increase their metabolite coverage. The answers to the question “Which type of metabolomics are you or your collaboration partners performing?” also indicated that more than half of the respondents rely on more than one method, which includes the use of several column selectivities.

As in routine laboratory medicine, the most common matrices for metabolomics experiments reported were serum and plasma, followed by urine and whole blood, together with dry blood spots.

Applications of clinical metabolomics

The objective of the second part of the survey was to gain a more detailed understanding of the applications of metabolomics by those who reported using it in their field of work. It was not surprising given the lack of commercially offered metabolomic solutions/platforms that research was the predominant activity reported, primarily for the purpose of discovering novel diagnostic or prognostic biomarkers that could ultimately be clinically translated. However, a great number of respondents reported the use of metabolomics for applied or routine clinical testing for diagnosis or prognosis.

The most prevalent areas of applications were metabolic diseases (primarily non-communicable diseases), inborn errors of metabolism, and a wide range of endocrine diseases. Current applications of metabolomics for patient monitoring were primarily for personal health/nutritional assessment and risk stratification for personalized prevention.

Applications to clinical research: from biomarker discovery to improved diagnostics, prognostics and personalized health monitoring

In our survey, 69 % of respondents apply metabolomics in the context of their research projects, consistent with their employment primarily by academic institutions, mainly university hospitals and research institutes and laboratories. The scope of the research projects in which metabolomics approaches are used ranges from fundamental biomedical research for obtaining mechanistic insights (i.e. mode of action) to applied clinical research for biomarker discovery, for improved diagnostics and prognostics of a wide panel of metabolic diseases. This is in line with the recent recognition of altered metabolism as a hallmark of multiple metabolic diseases, ranging from inborn errors of metabolism to acquired non-communicable cardiometabolic diseases (e.g. obesity, diabetes, cardiovascular diseases), to different cancer types, and neurodegenerative disorders (e.g. Alzheimer's or Parkinson's disease and other related cognitive impairments) [3]. Indeed, a wide range of clinical research studies, including prospective human population or epidemiological studies, and clinical intervention studies, have reported significant metabolic alterations associated with cardiometabolic risk factors such as age, sex, changes in anthropometric parameters, adiposity, diabetes or dyslipidemia markers [9, 10]. However, it remains unclear from a mechanistic standpoint whether the perturbed metabolism, particularly energy metabolism including energy utilization and storage, as well as metabolic signaling, should be seen as a cause or a consequence of disease onset, development, and progression [11–13]. Despite the lack of true mechanistic insights, there is substantial evidence that identified sets of metabolite (including lipid) markers can significantly improve the sensitivity and accuracy of disease diagnosis and prognosis (compared to traditionally applied prediction models essentially based on cholesterol and blood pressure) [14–16]. Improved diagnostics and prognostics in routine clinical practice are prerequisites for the more precise approach to patient and health monitoring that is the promise of precision medicine [2, 17–19].

Current applications in clinical research 1: metabolic diseases and endocrinology

The respondents of the survey have overwhelmingly highlighted metabolic diseases and endocrine disorders as fields where metabolomics contributes significantly to both clinical diagnostics and prognostics. Given that these conditions bear substantial consequences for public health and exert a considerable influence on overall healthcare expenditure, it logically follows that metabolomics is anticipated to play a pivotal role in their understanding and management. For instance, once the diagnosis of type 2 diabetes is established, clinicians rely on various biomarkers to predict and avoid future complications, like cardiovascular diseases and microvascular disorders including retinopathy, neuropathy, and nephropathy. Metabolomics approaches are thus expected to be major contributors to a more personalized approach to patient care [20–24]. In clinical practice, the simultaneous measurement of multiple steroid hormones, mainly by LC-MS/MS approaches, has already helped to provide a more holistic understanding of very complex adrenal disorders, especially in children, where symptoms might be less suggestive and sample volumes are reduced [25]. Another compelling illustration of the pivotal role metabolomics plays in endocrinology is exemplified by its thorough exploration of vitamin D metabolites. This in-depth analysis seeks to enhance our comprehension of the intricate interactions among these metabolites, elucidating the dynamic roles each one plays. This nuanced understanding not only contributes to unraveling the complexity of vitamin D metabolism but also facilitates the diagnosis of certain diseases, such as those associated with CYP24A1 mutations [26, 27].

Current applications in clinical research 2: inborn errors of metabolism

In the field of inborn errors of metabolism, targeted metabolomic approaches began to be used more than a decade ago [28]. Due to the nature of significant changes (up to 3 orders of magnitude) in biofluids, metabolomics offers an effective tool for the diagnosis of both known diseases and for the discovery of previously unrecognized ones. As one of the few areas of laboratory diagnostics in which metabolomics has already proven value, this tool has now become part of the routine diagnostic process, both at the level of targeted [29] and non-targeted [30] analysis, where it plays a growing role. A particularly important application is neonatal screening based on mass spectrometry. Because of the way these data have classically been evaluated (concentrations of individual

metabolites), newborn screening can be considered as related to metabolomics, although it relies on a classical quantification of a limited number of metabolites (usually from a few to higher tens of amino acids, acylcarnitines and others; depending on national programmes).

Current applications in clinical research 3: oncology

By providing valuable new insights into the metabolic features associated with cancer risk and progression, metabolomics has emerged as a promising tool in determining the prognosis of oncological diseases. Compared to other analytical techniques such as routine molecular diagnostics, metabolomics has the advantage of providing a more holistic view of the complex and heterogeneous nature of cancer. While previous research has already correlated metabolic profiles with cancer prognosis, treatment efficacy, and early diagnosis of various cancers (e.g. gynecological and lung cancers) [31, 32], it is expected that technological advancements (e.g. LC-MS and single-cell analysis) will further facilitate the identification of specific biomarkers and provide unprecedented opportunities to investigate cancer metabolism at a cellular resolution [33]. Notwithstanding its clinical promise, the practical utility of metabolomics in cancer prognosis is still an area of active research. The next necessary step will be to thoroughly validate and translate these promising results of hundreds of research studies into routine laboratory practice.

Current applications in clinical research 4: cardiometabolic risk

Cardiometabolic disease (considered here as combined atherosclerotic cardiovascular disease [ASCVD] and diabetes mellitus [DM] linked metabolically by insulin resistance and dyslipidemia) is the leading cause of death and disability worldwide. Its prevention via personalized risk identification and tailored treatment is therefore a very high clinical priority. Given the complexity of the molecular mechanisms underlying the pathophysiology of cardiometabolic disease, metabolomic profiling is considered capable of greatly improving upon the prognostic performance of standard cholesterol and glycemia screening [34–37]. Thousands of papers have been published in the last five years describing research addressing this clinical promise, but specific examples of successful clinical translation of metabolomic assays that demonstrably improve the standard of care are rare. One reason is that, so far, biomarkers of postulated

novel mechanistic pathways independent of those addressed by conventional risk factors have not been found. In one example, a comprehensive study using untargeted NMR metabolic profiling identified multiple NMR features associated with subclinical atherosclerosis and future ASCVD events, but these largely overlapped with known risk factors [38]. There is, however, good reason to be optimistic that metabolomics will ultimately contribute meaningfully to clinical cardiometabolic risk assessment and management. Notably, many respondents in our survey reported they are already using metabolomics for either cardiometabolic diagnostic (n=27) or prognostic (n=25) purposes.

To illustrate a possible approach for translating omics-based cohort studies into assays for CVD risk stratification, one could look to the success story of ceramides. Ceramides are circulating bioactive lipid metabolites that were shown to be associated with increased CVD risk. Over the span of about a decade, initial discovery studies deploying a range of lipidomics approaches were translated into an assay comprising four [39] to six [14] ceramide molecular species whose concentrations in human blood correlate strongly with risk of death from CVD [27]. This panel of ceramide molecular species has been licensed by leading diagnostic providers [40]. However, the additional benefit of using a ceramide-based risk management over traditional ones remains to be demonstrated [41].

Current applications in clinical research 5: clinical toxicology

Clinical and forensic toxicology usually relies on the direct detection and/or quantification of xenobiotic substances (including medications, drugs, drug of abuse, metals, environmental toxins, and other chemical agents). However, as for the endogenous metabolites, this approach can be an analytical challenge, due to their various physicochemical properties, their low circulating concentrations, or their short detection window in usual matrices through metabolism and/or rapid elimination. The metabolome changes in response to external stimuli, making metabolomics a powerful complementary tool to those currently used in toxicology to confirm an exposure and/or an effect and/or a toxicity due to xenobiotics [42]. In the case of toxicological analysis of live patients, this is clinically relevant to improve the diagnostic sensitivity and detection window indicating drug consumption or intoxication due to acute drug intake (e.g. new psychoactive substances [43]) or drug-facilitated crime context (e.g. GHB [44]). In addictology, metabolomics is a potential alternative approach for (1) diagnosing recent consumption (2) monitoring consumption, withdrawal or relapse, and thus

predicting the effectiveness of treatment [45]. In addition to the identification of biomarkers, other metabolomics studies were designed to evaluate acute and chronic toxicity mechanisms. In the post-mortem period, the metabolome undergoes time- and location-dependent modifications through degradation, accumulation or redistribution of metabolites, depending on their physico-chemical characteristics. Post-mortem metabolomics have been suggested as a potential tool for discovering new biomarkers that may assist in death investigations, and notably to improve interpretation of the causes of death or for estimating the post-mortem interval (time elapsed since death) [46, 47]. Large-scale post-mortem metabolomics has proven its ability to discriminate between different groups based on their metabolic fingerprints. In preliminary research, metabolomics was successfully applied to post-mortem samples of selected patients for screening purposes. However, this is still awaiting application in routine diagnostic practice [48].

Future of clinical metabolomics – the view of the community

The final part of the survey was devoted to the anticipated future prospects for clinical metabolomics and lipidomics by the laboratory medicine community. Besides the expected increase of the number of clinical metabolomics assays within the next five years, benefits are expected in all areas of laboratory medicine: diagnostics, prognostics, biomarker discovery, precision medicine, monitoring and clinical intervention. In particular, major benefits from metabolomics are foreseen in metabolic diseases, inborn errors of metabolism, oncology toxicology/pharmacology and neurodegenerative diseases.

However, despite such optimism, it is necessary to acknowledge and address a number of difficulties that lie ahead. Technical complexity, lack of standardization of methods and evaluation, lack of expert availability and the limited commercial solutions were reported as the main limitations for the future development of clinical metabolomics. In particular, laboratories face the major challenge of financing the purchase of devices and securing reimbursement of the assays by insurance companies or healthcare payers.

Limitation of the survey

This survey-based review aimed at the global evaluation of the metabolomics applications in the clinical environment and their potential for translation to clinical routine, with the emphasis on main hurdles and expectations (i.e. clinical utility). Thereby, the assessment of best practices, including

the quality assurance and quality control strategies was out of the scope of this report. This important aspect of metabolomics workflow has been previously addressed in separate reviews and the authors encourage readers that are new in the field to familiarize themselves with these concepts (e.g. [49–51]). In the same line, the lipidomics applications could be evaluated separately, due to obvious emerging potential.

Conclusions

Overall, the authors were positively surprised with the general interest this survey generated as well as with the high numbers of participants who are already involved in metabolomics activities. Although a definition for metabolomics was provided at the beginning of the survey (see above), the frontier at which metabolomics begins and classical clinical chemistry panels ends is not defined. This can explain the high number of participants reporting the use of metabolomics as routine assays. Nevertheless, we are witnessing a number of advanced technologies (mostly based on mass spectrometry) capable of acquiring metabolomics data being established in clinical laboratories. So far, only a limited number of groups have managed to successfully transition from research use to *in vitro* diagnostics (IVD) assays. A decisive aspect for the successful implementation of these types of assays, based on the determination of multiple parameters, will most certainly lie in the ease of interpretation of the results for clinical decision making.

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