Metabolomics in Sepsis and Its Impact on Public Health

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Abstract

Sepsis, with its often devastating consequences for patients and their families, remains a major public health concern that poses an increasing financial burden. Early resuscitation together with the elucidation of the biological pathways and pathophysiological mechanisms with the use of “-omics” technologies have started changing the clinical and research landscape in sepsis. Metabolomics (i.e., the study of the metabolome), an “-omics” technology further down in the “-omics” cascade between the genome and the phenome, could be particularly fruitful in sepsis research with the potential to alter the clinical practice. Apart from its benefit for the individual patient, metabolomics has an impact on public health that extends beyond its applications in medicine. In this review, we present recent developments in metabolomics research in sepsis, with a focus on pneumonia, and we discuss the impact of metabolomics on public health, with a focus on free/libre open source software.

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Introduction

Sepsis is a clinical syndrome with substantial heterogeneity. As a result, diagnostic definitions are based on non-specific criteria being often under revision [1, 2]. In pathophysiological terms, sepsis is perhaps best defined as the clinical syndrome produced by an exaggerated, uncontrollable immune response to an infection [3]. Sepsis and its more severe forms, such as septic shock, may lead to organ dysfunction, organ failure, and death [4]. Although its mortality has decreased over the last years, mainly due to the application of bundled early goal-directed therapy [5], sepsis, with its often devastating consequences for patients and their families, remains a major public health concern that poses an increasing financial burden. Indicatively, a mean intensive care unit (ICU) cost per patient of more than USD 27,000 and an average length of stay in the ICU and the hospital often extending beyond 5 and 20 days, respectively, illustrate the burden imposed on the patients, their families, and national health care systems worldwide as well as the need for early diagnosis and personalized treatment of sepsis [6–8].

On the other hand, “-omics” technologies have revolutionized our screening and prevention programs, al-

lowing for early diagnosis, proper treatment, and minimization of costs across almost the whole spectrum of diseases. “-omics” are thus widely considered an indispensable, organic part of modern public health [9, 10]. Apart from their benefit for the individual patient, “-omics” technologies have an impact on public health that extends beyond their applications in medicine. Being essentially a subspecies of big data, “-omics”, from genomics and transcriptomics to proteomics and metabolomics, are commonly dependent on free/libre open source software (FLOSS), often developed by the researchers themselves, for the analysis and interpretation of vast amounts of complex data. To a certain degree, the effect of “-omics” on public health is mediated by the extended use of FLOSS and the formation of commons arrangements within the research community.

The application of “-omics” technologies in sepsis research has already started unraveling this highly heterogeneous clinical syndrome with the development of new diagnostic tools and therapies nowadays relying to a great extent on the further elucidation of biological pathways with the use of integrated “-omics” [11].

In this review, we focus on the potential of metabolomics as an “-omics” approach that can shed light on the underlying biology and pathophysiological mechanisms of sepsis. After a short discussion of other “-omics” approaches in sepsis and their restrictions, we describe the main techniques and methods in metabolomics, focusing on the use of FLOSS. We then present recent work in the field, focusing on pneumonia, that can help advance our knowledge of sepsis biology and pathophysiology. In the end, we briefly discuss the impact of metabolomics on public health and present challenges for future research in this field.

The “-omics” Cascade in Sepsis Research

Developments in molecular biology opened the road from genetics to genomics, with the Human Genome Project and the International HapMap Project both providing new avenues for advances in the field of biomedical sciences and biotechnology [12, 13]. Studies at the level of the genome have disruptively increased our knowledge in many areas. Most notably in autoimmune diseases, such as Crohn’s disease, but also in diabetes mellitus and cardiovascular diseases, genome- and exome-wide association studies (GWAS) have led to the correlation of certain single-nucleotide polymorphisms (SNPs) with certain phenotypes, revealed the existence of

shared risk loci among entities, highlighted differences in genetic susceptibility across ethnic populations, and, with pharmacogenomics, even contributed to drug safety and efficacy testing [14–20].

Despite the incontrovertible successes of genomic technologies in autoimmune, metabolic, and cardiovascular diseases, genomics research has delivered comparatively moderate gains in the elucidation and treatment of the septic syndrome. SNP genotyping of several genes related to lymphocytes, Toll-like receptors, and ligands for pathogens’ elements have indeed enriched our knowledge of the biological background of sepsis [21–25]. However, the pathophysiological relevance of those findings remains unclear and the use of this knowledge in clinical practice, with the notable exception of neonatal ICUs, is still limited [26]. One of the reasons for the difficulty to put such findings in a pathophysiological context is that sepsis, in contrast to autoimmune entities, such as Crohn’s disease, is a heterogeneous syndrome, in effect the end of numerous, disorganized activated pathways at multiple levels between the genome and the phenome. This heterogeneity, coupled with the high number of patients required for a GWAS to identify loci conferring a modest risk, exaggerates the difficulties associated with sepsis definition and makes the recruitment of patients truly challenging [27]. Furthermore, as the development of sepsis requires an insult from a pathogen, the exertion of risk allele effects cannot be identified before the host-pathogen interaction has started. Besides, controls in GWAS in sepsis are often healthy subjects who, even if they carry the alleles conferring a risk of sepsis development, have not undergone the required precipitating event (i.e., they are not infected yet). These attributes of genomics research in sepsis may result in possible biases and perturbation [28, 29]. In addition, the genomic approach alone has left many questions unanswered such as, for example, what is the role of epigenomics [30], which genes are being activated during the course of a disease, how the host-pathogen interaction changes the expression of certain genes [31, 32], and which gene products are indeed being produced and modified as a response to an insult. [33]. Studies further down from the genome to the phenome, at the level of the epigenome, transcriptome, and proteome, have been, to some extent, successful in complementing and expanding our knowledge of disease pathophysiology [34–39]. Nevertheless, as transcriptional and translational elements exist in high numbers of variants and isoforms and are dynamically altered (with unknown time lags) through the interaction with the insulting pathogen, the results of transcriptomics

and proteomics research in sepsis, though promising, need to be confirmed before they are applied at the bedside [40].

The Metabolomics Approach

Metabolomics, “the quantitative measurement over time of the metabolic responses of an individual or population to drug treatment or other intervention” is a promising area of research, further down in the “-omics” cascade between the genome and the phenome, that could be particularly fruitful is sepsis research [41]. Although, historically, the term metabolomics has not been interchangeable with the less frequent “metabonomics,” both terms have come to refer to the comprehensive study of the metabolites of a living system (i.e., the metabolome), either naturally occurring or as a response to a particular set of conditions [42].

Investigation of the metabolome ranges from methods relying on pattern recognition, such as metabolic fingerprinting, to quantification methods, such as metabolic targeting and metabolic profiling. Institutionalization [43], a growing number of relevant studies, an increasing economic impact [44], and a very active community of FLOSS users and developers active in metabolomics research are signs of the increasing importance of metabolomics as an “-omics” technology.

Being a complex function of intra- and extracellular processes and encompassing information from many regulatory processes (e.g., from epigenetic regulation of transcription to allosteric regulation of proteins), the metabolome provides integrated information further down from the genome to the phenome. On the other hand, as already mentioned above, sepsis is in effect the result of the complex interactions between the host and the insulting pathogen. Sepsis-induced alterations in the genome, transcriptome or proteome are reflected in changes in the concentration of the small molecules and chemicals in biological fluids and tissues [45]. These changes can be accurately measured and analyzed, thus providing a readout of the fluctuations of metabolites and other intermediate products of metabolism in the setting of an environmental insult, such as a severe infection. Such studies may reveal novel biological pathways extending between the genome and the phenome and offer opportunities for diagnostic and therapeutic interventions. Technical developments in the last decade have allowed accurate profiling of diverse intra- and extracellular substances, rendering metabolomics “the final piece of the -omics puzzle” [46].

Although analytical chemistry (“the laboratory”) has been an indispensable part of (and to some extent has defined) biomedicine for more than a century [47], improvements in the sensitivity and specificity of the analytical techniques used in metabolomics allow for the simultaneous measurement of hundreds of metabolites in a variety of sample fluids [48]. More importantly, advances in the Information and Communication Technology (ICT) sector in the last decades (with early implementation in interpretation of the then rapidly accumulating biochemical data) [49] allow us to interpret the results in biologically meaningful ways [50].

Specifically in sepsis, metabolomics is a very promising field of research for a number of reasons. In contrast to genomics and proteomics, the impact of environmental perturbation on the metabolome results in changes that are highly dynamic and occur over shorter periods of time [51]. Furthermore, the number of metabolites appears to be considerably lower than the number of variant gene expression profiles and protein isoforms (42,003 metabolite entries until January 2017) [52, 53]. Taken together, these characteristics render metabolomics unique, both as a research approach (design of clinical trials for the discovery and validation of biomarkers) and as a powerful tool in the clinical practice (real-time monitoring of time-dependent metabolite changes). Particularly in critical illness, the plethora of data derived from metabolomics studies could potentially change the landscape in biomarker discovery by enabling disease detection and monitoring with the use of not just a single biomarker but a compilation of them [45, 54].

In the following section, we briefly address the metabolomics techniques and methods with an emphasis on data analysis and the use of FLOSS.

Metabolomics Techniques and Methods

Metabolomics techniques provide a “snapshot” of the many small molecules (such as nucleotides, amino acids, lipids, etc.) that constitute the metabolome. Designing experimental studies in metabolomics requires consideration of several issues. Targeted metabolomics, where a fixed subset of certain metabolites (sometimes up to 500) is studied, is particularly useful in testing a specific hypothesis. The use of internal calibration in targeted approaches confers measurement accuracy. Conversely, untargeted profiling, where all metabolites in a sample are measured, is better suited for hypothesis-generating studies. Identification of novel metabolites and the lack of bias

are the main advantages of untargeted metabolomics. As far as the analysis of the data derived with each strategy is concerned, in general, analysis of untargeted data is often more demanding due to detected signals from still unidentified metabolites. Other important issues include the choice of the appropriate population (cell cultures, animals, humans, etc.) and sample (fluid, tissue, etc.), the decision to analyze intra- or extracellular metabolites, and, importantly, statistical considerations. Irrespective of the above design options, because of the nature and variety of the physicochemical properties of small molecules, the methodologies and analytical techniques employed are usually multiple, with one-dimensional (1-D) proton (^1H) nuclear magnetic resonance (^1H -NMR) and mass spectroscopy (MS), coupled with multivariate analysis, being the main analytical platforms [50].

Nuclear Magnetic Resonance

This technique involves the generation of magnetic fields with the use of powerful magnets. Briefly, a sample in a nuclear magnetic resonance (NMR) glass tube in a magnetic field is excited with a radio frequency pulse. Alternation between the lower and higher energy spin states of the electrons generates a resonance which is unique for every substance, depending on its chemical structure. After subtraction of perturbation (e.g., water signals) this substance-specific NMR response is registered, processed, and displayed in the form of a peak across a spectrum. The area under the peak represents the relative concentration of the respected metabolite compared to an appropriate reference signal, thus allowing precise quantification. As the NMR response is unique for every small molecule, it is possible to calibrate the magnet in order to reproducibly target certain compounds, as in the case of targeted metabolomics. Another main advantage of NMR is that the technique is non-destructive, thus allowing for retesting if necessary. Furthermore, it requires minimal sample preparation, is relatively quick, and has a sensitivity that increases with the use of more powerful magnets (ranging between mM/L and $\mu\text{mol/L}$) [55, 56]. However, detection limits and quantification unreliability in the area below $\mu\text{mol/L}$ necessitate the use of more sensitive techniques, such as MS.

Mass Spectrometry

Identification of metabolites with MS relies on the detection of the mass-to-charge ratio (m/z) and the relative intensities of ionized compounds. Briefly, molecules bombarded with charged elements are split into fragments separated within an (electro)magnetic field and sorted along their m/z ratio. The results are then differ-

entially displayed according to the relative abundances of fragments with the same m/z ratios. Liquid chromatography or gas chromatography, which improve mass separation, often precede MS in the setting of metabolomic studies.

Compared to NMR, MS-based platforms are more laborious and more destructive for the sample; however, they have a greater sensitivity. In general, the more sensitive MS provides information about the structure of the detected compound, whereas the more accurate NMR provides certainty and quantification [46].

Data Analysis and Biological Interpretation

The relative advantages and disadvantages of the two platforms should be taken into account in the design of metabolomic studies and often both approaches are employed. A common characteristic is that both analytical platforms generate vast amounts of multidimensional data that need rigorous analysis. Metabolomic data analysis ranges from platform-specific operations, such as data processing, often performed with the use of proprietary, built-in software, to platform-independent statistical analysis and biological data interpretation, where FLOSS plays an ever-increasing role. Here, we discuss briefly the main considerations regarding metabolomic data analysis with an emphasis on the use of FLOSS.

Statistical Analysis

Due to its amount, complexity, and characteristics (many variables for few observations), metabolomic data requires the use of multivariate statistical analysis. Multivariate methods can be broadly divided into unsupervised tests, such as principal component analysis, routinely used to identify outliers and detect natural clustering within the studied data set, and supervised methods, such as partial least squares analysis, best suited for the construction of predictive models. Results of untargeted profiling including detected but still unidentified metabolites can also be subjected to statistical analysis as the latter uses absolute or relative values (i.e., intensities) even if they do not correspond to known spectra. Statistical analysis is often performed with the use of commercial multivariate analysis packages; however, FLOSS packages are also available. In essence, and irrespective of their complexity and the tools used for the analysis, the results of statistical analysis in metabolomics display associations between metabolites and certain phenotypes in health and disease.

Data Interpretation

Significant associations of metabolites with phenotypes need to be meaningfully aggregated and interpreted in order to provide biological insight. Putting associations in context (usually by accommodating them into a certain perceived setting) may be challenging [57]. It includes mapping observed changes of known metabolites onto biological pathways as well as estimating the comparable importance of the various pathways using methods already employed in other “-omics” approaches, such as enrichment analysis. A limitation of pathway mapping is the relatively low number of metabolites included in the analysis, basically due to the lack of reliable metabolite identifiers. Generation of automated annotations [58] and construction and visualization of metabolite networks [59] attempt to overcome these challenges. Based on the observation that metabolites that are functionally related also change in a similar manner over time or over the course of an event that affects this function, data-driven analysis is another effort to include detected but still unidentified metabolites into pathway analysis [60].

Data interpretation relies largely on free/libre open source software platforms that allow for mapping of metabolites onto biological pathways and visualization of the constructed networks. This is a common characteristic between metabolomics and the other “-omics” technologies. In the following paragraphs, we present in brief the relationship between metabolomics and FLOSS.

Free/Libre Open Source Software

Technological developments have led to enormous advances in metabolomics instrumentation. To gain a sense of proportion, the first instrumentation of MS had a mass resolving power of 130 whereas nowadays the latter may amount 200,000 [61]. The potential of modern instrumentation, which produces vast amounts of complex data, poses a big challenge to our ability to deal with it. Even commercial, instrument-specific, built-in software is not always capable of taking full advantage of the capacity of the hardware. Pioneers in biomedicine (e.g., molecular biology, biochemistry, etc.) and bioinformatics, especially in the post-World War II period, were often obliged to develop their own software in order to make sense of data [62]. On the other hand, management, analysis, and effective exploitation of such data require collaboration between researchers. Hence, it may be argued that networking and sharing of knowledge, in the form of open-source codes, has almost been an imperative in the field of big data, such as metabolomics. Within this context, the use of FLOSS has been an integral part of metabolomics research.

There are several issues regarding the use of FLOSS in metabolomics. Instrument-specific, built-in software saves data in a proprietary format so that export of data in open, common formats, even when possible, may lead to loss of data. Abandonment and fragmentation of efforts, difficult-to-use interfaces, multiple bugs, and, more importantly, a lack of both software validation and technical support are some of the disadvantages of open-source platforms as compared to proprietary software. On the other hand, FLOSS platforms may have an edge over proprietary solutions for a number of reasons. Availability and accessibility of the source, as well as a very active community (mainly consisting of the researchers primarily involved in “-omics” research), allow for open scrutiny and timely fixing of bugs, rendering the argument of a lack of technical support rather obsolete. The lack of hidden costs for implementation and learning makes FLOSS an attractive choice in terms of budget, thus lowering the entry barriers in metabolomics research [61].

Reproducibility of results is a constitutive element of science. In the field of metabolomics, results highly depend not only on measurements of events but also on the assumptions used and the software and algorithms employed for their interpretation. As Jonathan Buckheit and David Donoho put it, “An article about computational science in a scientific publication is not the scholarship itself, it is merely advertising of the scholarship. The actual scholarship is the complete software development environment and the complete set of instructions which generated the figures” [63]. Reproducible research refers to freely available data and methods used in a research article and here FLOSS, with its open algorithms, has a place.

The relationship of metabolomics with FLOSS also has implications for public health, as we will see in the relevant section. However, the impact of metabolomics research on public health in the case of sepsis is primarily mediated by its tangible, direct effect on diagnosis and treatment. In the following section, we present recent work in metabolomics research in sepsis, focusing on pneumonia.

Metabolomics in Sepsis

Lactate, a metabolite formed from pyruvate in the cytosol as part of glycolysis, has been one of the most frequently used biomarkers of tissue hypoxia in sepsis [5]. Current evidence challenges this dominant view and sug-

gests that lactate metabolism during sepsis is a non-linear, complex process that needs to be further investigated [64, 65]. Irrespective of the value of lactate as a biomarker of tissue hypo-perfusion, this controversy only underlines the dynamic of current research in the field of metabolomics and how the latter could shed light on the complex biological pathways underlying the pathophysiology of sepsis.

Metabolomic studies in sepsis have primarily focused on sepsis-associated mortality [66, 67]. Both NMR and MS have been employed in the study of several metabolites in different biological fluids and tissues, mainly blood (serum and whole blood), bronchoalveolar fluid, and urine, in animal models as well as in humans [68–70]. Study design strategies involve different approaches, ranging from targeting certain pathways [71] to studying metabolite classes and mitochondrial dysfunction [72, 73].

Metabolomic profiling of patients with sepsis has identified a series of different metabolites that consistently correlate with increased mortality. Integrated analysis of outcomes in a prospective, observational trial of 1,152 subjects with suspected community-acquired sepsis revealed metabolic derangements with consistently higher values of certain metabolites in the group of non-survivors [66]. Despite this consistency regarding individual metabolites, network models developed to predict death on the basis of a group of metabolites have implicated different, non-overlapping sets of metabolites. Such discrepancies underline the role of and the challenges for the statistical analysis posed by the voluminous, complex data produced by the analytical methodology [74]. On the other hand, these results also highlight the potential and the difficulties of metabolomics in predicting sepsis mortality [75].

Sepsis is a time emergency, where survival is reported to decrease by 7.6% with every hour of delay in the initiation of therapy [76]. Especially the delay in the onset of the proper antibiotic therapy has been recognized as a major risk factor for mortality [77]. Aggressive, general, bundled measures, such as an early start of empiric antibiotic therapy, adequate source control, and hemodynamic and respiratory monitoring and support are cornerstones of sepsis management and essentially reflect the fact that sepsis is not a disease but rather a syndrome [5]. Nevertheless, the heterogeneity of the septic syndrome should also prompt adjustment of treatment to the precipitating factor. This is of particular importance in cases of unknown underlying infections, and here metabolomics seems to have a place. A retrospective analysis

of 406 patients, in which a total of 186 metabolites were determined by liquid chromatography tandem MS, identified specific metabolites that correlated with certain infections and could eventually be used for discrimination of the different triggering factors. In this study, the glycerophospholipid lysoPCaC26:1 identified patients with community-acquired pneumonia (CAP) and sepsis (as compared to patients with sepsis due to other infections) whereas putrescine, a metabolite produced by the breakdown of amino acids, was associated with unfavorable outcomes in this particular group of patients [78]. Another recent study used an untargeted approach to study the metabolome, in plasma samples, of 142 patients with CAP and 97 controls. Global analysis of the metabolome identified 13 lipid metabolites that could discriminate between CAP cases and non-CAP controls, and 2 metabolites that were associated with increased mortality [79].

CAP remains a dominant cause of hospitalization, morbidity, and mortality. Mortality rates among patients requiring admission to an ICU exceed 40% in multicenter cohort studies, and CAP complicated by sepsis is an emergency than needs to be timely recognized and treated [80]. Clinicians use severity assessment tools in order to stratify patients according to the severity of their disease. However, these tools have several problems that limit their efficacy, such as a compromised validity of studies due to poorly selected patients and the use of many different end points. Especially relevant for CAP and CAP-associated sepsis is that the use of different scores may lead to different clinical decisions. The following example illustrates the problem with the conventional, clinical assessment tools: a 60-year-old patient with CAP, a respiratory rate of 26 breaths/min, a blood pressure of 95/65 mm Hg, BUN of 18 mg/dL, and a normal mental status is being evaluated in the emergency department. According to the validated prognostic model CURB65 score (developed for pneumonia), this patient has an estimated mortality of 0.6% and could be managed on an outpatient basis. When evaluated with the use of qSOFA (the newest score for patients with suspected sepsis in the emergency department), the same patient has sepsis and should be probably transferred to the ICU [81, 82]. Notably, the newly developed qSOFA score seems to fail validation and “should not replace general early warning scores when risk-stratifying patients with suspected infection” [83].

Machine learning approaches have also been employed, complementary to clinical tools, in developing predictive models for sepsis; however, reliable diagnostic and predictive tools remain elusive [84].

From the information above, it is clear that current diagnostic tools need to be complemented with faster and more accurate methods that take into account the complex interactions between the host and the pathogens. The role of metabolomics in this case is illustrated by the following examples. *Streptococcus pneumoniae* is the most frequent infectious agent across the whole spectrum of patients with CAP (outpatients, hospitalized, ICU) [85], whereas *Staphylococcus aureus* is, together with *Pseudomonas aeruginosa*, the most frequent isolate in cases of hospital-acquired pneumonia (HAP) in ICU [86]. Quantitative metabolomics with the use of NMR in urine samples of patients with CAP have revealed a distinct metabolic profile for pneumococcal pneumonia as compared to viruses and other bacterial agents [87]. Furthermore, targeted metabolomics in urine samples have revealed early differences between the metabolic responses induced by *S. pneumoniae* and *S. aureus* in mouse models with lung infections [88]. Nearer to the patient, a prospective, cross-sectional cohort study involving 93 patients used exhaled breath metabolomics for the diagnosis of pneumonia in intubated and mechanically ventilated patients. Exhaled breath contains gas phase metabolites, called volatile organic compounds, produced by the host and the insulting bacteria. Thermal desorption with gas chromatography coupled to MS (TD-GC-MS) identified different sets of volatile organic compounds that discriminated, with moderate accuracy, between ventilated patients with CAP and ventilated patients with HAP as well as between ventilated patients with pneumonia (CAP or HAP) and ventilated patients without pneumonia. In this study, 1-propanol, a metabolite, was consistently detected in both patients with pneumonia and patients with colonized airways without signs of acute infection. 1-Propanol is a primary alcohol product of many fermentation processes. It is also produced by *Escherichia coli*, probably as a means to prevent the growth of other pathogens. Taken together, these facts suggest a potential role of 1-propanol as a biomarker for bacterial presence and growth and should prompt further investigation [89]. These findings stress the potential of metabolomics as a tool for disease diagnosis and monitoring in pneumonia and pneumonia-associated sepsis that adds value to the current practice.

The utility of metabolomics in sepsis also lies with the identification of metabolites from certain pathways in bacteria that could reveal potential antimicrobial targets, thus changing not only the diagnostic but also the therapeutic landscape [90]. A recent study used quantitative 1H-NMR spectroscopy to study the effects of 5 antibiotic

classes to the metabolic response of *E. coli* cultures. Metabolic footprints were class specific when *E. coli* cell cultures were treated with antibiotic agents acting intracellularly. However, the use of antibiotics targeting the cell wall produced distinct, antibiotic-class-specific, metabolic responses. Remarkably, the mode of action of 3 different antibiotics (treptomycin, tetracycline, and carbenicillin) could be correctly predicted [91]. The studies outlined above illustrate the fact that the therapeutic potential of metabolomics in metabolic resuscitation in sepsis is gaining recognition [92, 93].

Nevertheless, despite this progress, there are several issues that limit the use of metabolomics in clinical practice. Unfortunately, a series of biomarker candidates have failed validation in confirmation studies. Furthermore, in a recent study, untargeted metabolic profiling of unrelated diseases yielded similar findings among patients with different diseases as compared to healthy controls. In addition, metabolites that were associated with specific diseases in this study were found to have already been associated with other diseases in the relevant literature. To overcome these confounding results and to increase the chances of successful validation, researchers suggest a design strategy that should also include, apart from healthy ones, controls with non-related diseases [94]. Another important limitation is the limited number of study participants and the large number of variables. With some of the metabolites still unidentified, interpretation and contextualization of results remain a challenge. The employment of new approaches, such as data-driven analysis, and the development of appropriate software solutions will lead to a better exploitation of the current hardware capabilities. Lastly, but equally importantly, the road between impractical, laborious, and time-consuming applications, such as MS, and the use of bedside sensors to accurately diagnose or exclude pneumonia remains long and warrants the necessary technological solutions [95].

Impact on Public Health

Being a component of epidemic preparedness and response support and a core element of informed public health action, “-omics” technologies, in general, have a well-known impact on public health [96]. However, the impact of “-omics” research on public health is not limited to the control of epidemics. In the specific case of sepsis-associated metabolomics research, its impact on public health is primarily mediated by its effect on disease

diagnosis, monitoring, and treatment. Development of composite biomarkers for sepsis, understanding of the relevant pathophysiological mechanisms, elucidation of pathogens' biological pathways, identification of novel antibiotic targets, tailored drug treatments, and therapeutic monitoring are expected to realize personalized medicine, thus increasing survival rates and reducing costs for the treatment of patients with sepsis [97].

It has been argued that personalized medicine is an integral component of modern public health [98]. On the other hand, metabolomics has been characterized as a driver for personalized medicine in sepsis [99]. Taken together with the fact that sepsis, as mentioned above, is a global health concern, this underlines the significance of metabolomics for public health. More specifically, by complementing other “-omics” technologies, employment of metabolomics has the potential to radically transform the research and clinical landscape in sepsis by enabling effective risk stratification and diagnosis [100], prognosis [101], and, with pharmacometabolomics, even targeted therapeutic approaches [102].

However, metabolomics, as is the case with the other “-omics” technologies, is a horizontal technology that transcends nominal categories, so that developments in sepsis-associated metabolomics research may have a more general impact on public health. In effect, this means that findings of sepsis-related research can be transferred to other fields. For example, in an era with increasing numbers of antibiotic-resistant pathogens and a limited number of novel antibiotic agents, application of metabolic fingerprinting could complement our knowledge of the mechanism of action of new antibiotic agents with implications for drug discovery [91]. The relevance for public health becomes even more obvious if we take into account the multitude of septic infections affecting intensive fish farming (with the danger of the resulting epidemics), in an era where seafood is the highest-value globally traded food commodity [103]. The same holds true for farming, where food metabolomics play an ever-increasing role in the diagnosis and treatment of infected livestock [104].

But the impact of sepsis-associated metabolomics research on public health extends beyond drug discovery and personalized medicine as it increases the capacity of biomedical research in numerous ways. For example, a basic characteristic of “-omics” technologies is the production of vast amounts of data that need analysis and interpretation. Effective management, analysis, and exploitation of data require readiness for cooperation among researchers as well as other actors, a low level of

suspiciousness, and the willingness to share information with others. FLOSS is a prominent example of this new ethos. Due to the complexity and amount of data, researchers working with metabolomics (and other “-omics”) are often confronted with the need to customize the software used for data analysis and interpretation. As already mentioned above, FLOSS is an invaluable resource that allows for such customization. Researchers that develop, use, and freely circulate (free as in speech) [105] FLOSS for use in metabolomics research are essentially forming a modified commons arrangement (where commoners are the researchers/developers of the software, the latter is the shared resource, and the operating rules are those of the corresponding public license). Such commons arrangements transcend conventional national, academic, and industrial barriers. For example, researchers in a country can make use of FLOSS developed elsewhere, modify it according to their needs, and advance their research. By making it freely available, the added value conferred to the software can be used by others in an ever-enriching circle with obvious benefits. FLOSS, which is arguably an innate characteristic of “-omics” technologies, illustrates another aspect of the potential of metabolomics as an engine for biomedical innovation. In the real world, this potential is realized by the formation of arrangements that enable transactions based on metabolomic data [106].

Conclusion and Future Research Challenges

In summary, the rapidly growing field of metabolomics has the potential to change the clinical practice in sepsis. In contrast to genomics, transcriptomics, and proteomics, metabolomics targets a point in the “-omics” cascade even closer to the phenome, where possibilities (encoded in the genome) have already been materialized. Studies in sepsis suggest that metabolomics may lead to the discovery of composite biomarkers and contribute to the drug development process. Future work could focus on pursuing a better stratification of patients (e.g., early identification of non-responders, predisposing phenotypes, etc.) and enabling early recognition of challenging diagnoses, such as ventilator-associated pneumonia. To this end, employing an integrated, multi-omics approach would present a considerable challenge but also a huge opportunity for advances in the field. The impact of metabolomics on public health is primarily mediated by its effect on sepsis diagnosis, monitoring, and treatment. However, its relevance for the bioeconomy as well as the

extended use of FLOSS and the resulting formation of communities of researchers that cooperate with each other are also important effects of metabolomics. Here, future research should seek to map the landscape, quantify these effects, and suggest measures to overcome barriers to cooperation.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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