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*Review*

# Integrative metabolomics to identify molecular signatures of responses to vaccines and infections

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**Abstract:** Approaches to identification of metabolites have progressed from early biochemical pathway evaluation to modern high dimensional metabolomics which is a powerful tool to identify and characterize biomarkers of health and disease. While traditionally considered relevant in the context of classic metabolic diseases, immunometabolism has emerged as an important area of study as leukocytes generate key metabolites important to innate and adaptive immunity. Herein we discuss the metabolomic signatures and pathways perturbed during infection as well as vaccination. For example, changes in lipid and amino acid pathways (e.g., tryptophan, serine, and threonine) have been noted during infection while carbohydrate and bile acid pathways have shift upon vaccination. Metabolomics holds substantial promise to provide fresh insight into the molecular mechanisms underlying host response to infection and vaccination, and its integration with other systems biology platforms will add further impact to our studies of health and disease.

**Keywords:** metabolomics; vaccines; infections; integrative metabolomics; systems biology, diagnosis, response detection

## 1. Introduction

Metabolites are small molecules (e.g., 50 to 1500 Daltons) that are produced as a result of regulatory mechanisms and cellular processes or acquired from exogenous sources such as dietary, drug, and xenobiotic components [1]. Metabolites collectively provide a snapshot of the complex interplay between the genome, environment, and intermediary processes [2]. They play critical roles in biological pathways and serve as valuable bioindicators of changes in cell physiology [3].

As early as 1955, biochemical research provided the first perspective of a comprehensive cellular metabolome comprised of ~20 metabolic pathways [4]. As mass spectrometry evolved in the next few decades, it enabled a dramatic expansion in the range and detail of biochemical chart mapping [2]. From that time forward, the term "metabolomics" emerged as the final frontier of systems biology [5].

Metabolomics, as a study of the collection of metabolites in a biosample, uses high-throughput technology to assess these small molecules. With metabolism relevant to most if not all aspects of biology, this emerging and rapidly evolving technology measures a vast array of molecules with distinct properties [6]. Metabolomics identifies the entire collection of metabolites (e.g., lipids,

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carbohydrates, and amino acids) in a given sample and is a new and powerful approach for clinical diagnostics. While mapping of the entire metabolome is a challenge due to heterogeneity in molecular characteristics and the wide range of metabolite concentrations, development of several assay platforms such as liquid chromatography mass spectrometry (LC-MS) or nuclear magnetic resonance (NMR) based spectroscopy has enabled categorization of the metabolome into subsets based on molecular properties such as, polarity, structure, or function [7].

Within the framework of precision medicine and, in comparison to other systems biology approaches, metabolomics provides a nearly instantaneous physiological measurement. The metabolome rapidly responds to any even minor stimulation, rendering metabolomics a powerful approach to assessing responses to stress [8], nutritional changes [9], disease states [10], host-pathogen interactions [11] and especially short- and long-term metabolic effects mediated by infection [12] and vaccination [13]. Similarly, this systems biology detects changes in various pathways and products that span beyond multiple scientific fields and also even domains of life [14]. The multitude of metabolite changes across space and time and the snowball effect of these changes to downstream biological processes produces a wealth of data but also adds complexity to their interpretation, necessitating sophisticated detection methods, separation and analyses based on various structural characteristics and molecular differences. Of note, a large portion of the metabolome includes relatively underexamined lipid families [15]. There are thus still uncharted areas of the metabolome that may be key to the host response to infection [16-20] and vaccines [21].

In this review, we highlight metabolomics as an emerging tool for the identification of signatures and pathways as a response of the host to infection and vaccination. We will explore how metabolomics complements and integrates with other well-studied systems biology components to provide the most useful information of altered biochemical phenotypes. We will focus on how metabolomics determines the developmental, physiological, and pathological states of a biological system thereby representing a powerful tool for precision medicine.

## 2. Metabolomics combined with other 'omics as systems immunology tools

Systems biology approaches that focus on a single class of molecules such as transcripts (transcriptomics) or proteins (proteomics) can provide important but limited insights into the biological mechanisms of disease [3]. The addition of metabolomics provides insight into complementary and synergistic interactions across different cellular and molecular levels [22]. To provide a holistic understanding of a dynamic biological system, incorporating multi-omic variables addresses gaps in our current knowledge of disease pathogenesis and evolution and offers opportunities for early diagnosis, prevention and potentially treatment of disease [23].

Multiple approaches that integrate metabolomics and other systems biology data from the same sample enable a systematic dive in disease characterization. These approaches enable temporal associations between identified signatures and pathways and disease progression, or it the case of vaccinology, vaccine protection [24, 25]. Identification of disease phenotypes or vaccine efficacy markers using metabolomics has the advantage of not requiring elaborate amplification of nucleotides or protein digestion [6]. Assays that detect specific metabolites such as eicosanoid, arginine, and citrulline testing are currently employed for clinical use after being associated with immune regulatory pathways [26-28] as well as metabolic diseases [29-31]. Metabolite tests that are

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now routinely available in the U.S. for point of care testing include glucose, 1,5 anhydroglucitol, carbohydrates, lipid, and amino acid panels that can be readily ordered by clinicians [32]. Given such proof of concept examples, further expansion of clinical metabolic biomarkers may help fill the unmet need for biomarkers of infectious diseases and immunization.

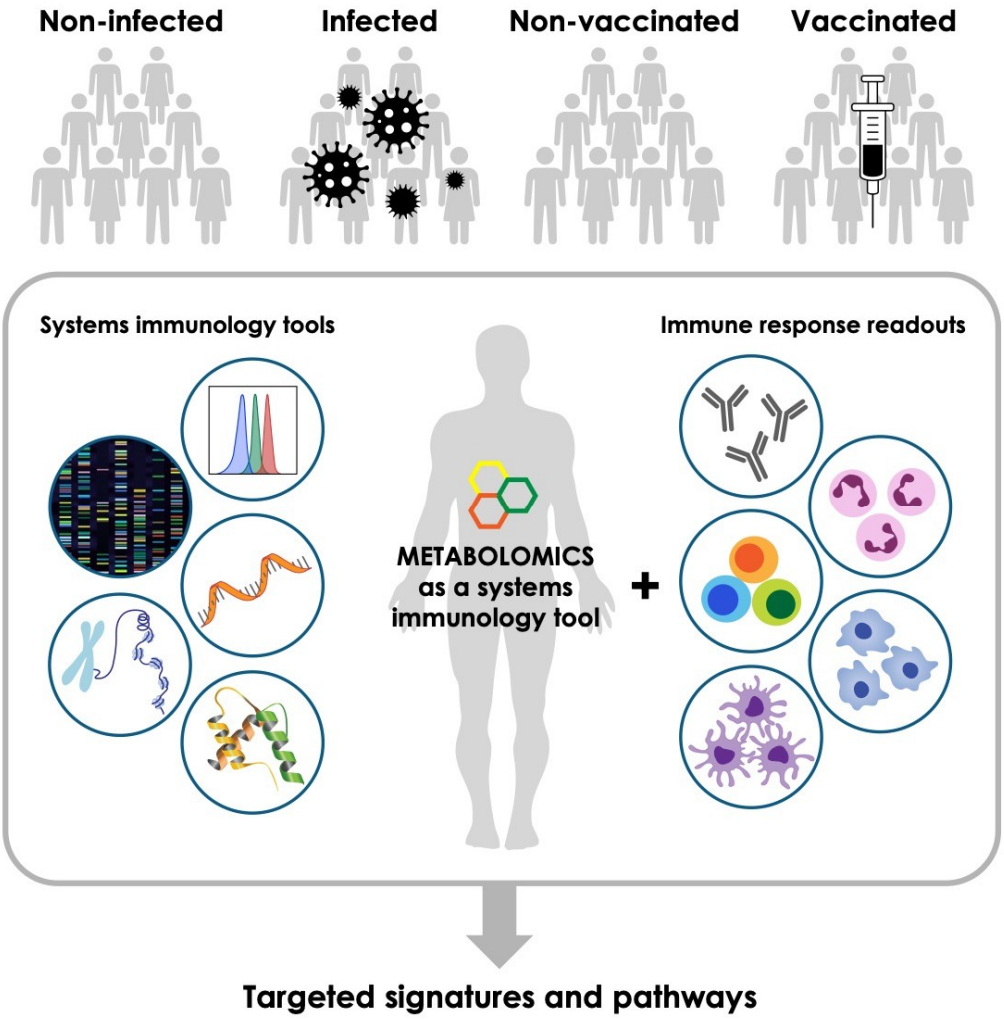


**Figure 1.** Metabolomics can complement other systems immunology tools for multi-omic integration with immunologic data to identify biomarkers of infection and vaccination.

From conventional immunology and reductionistic approaches, recent methodologies of systems immunology have enabled comprehensive analysis of multiple features in the immune system in parallel [33]. Systems immunology characterizes complex systems-wide measurements, assessing the relationship of each of the properties in the systems biology space with a known perturbation of the system such as vaccination or infection (Figure 1). Data integration, predictive modeling, and sophisticated machine learning algorithms facilitate hypotheses generation ultimately leading to new mechanistic insights in various biological processes.

Emerging as a high-throughput technology, mass spectrometry-based metabolomics defines molecular signatures of biochemical activity corresponding to distinct phenotypes. Overcoming blood volume limitations by employing a sample-sparing protocol, a recent multi-omic study of newborn peripheral blood demonstrated marked changes in numerous metabolic pathways across the

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first week of human life [34]. These included changes in steroids and carbohydrate metabolites that may play important roles in neurodevelopment, cell proliferation, and uptake of nutrients in early life [34].

The availability of mass spectrometry (MS)-based analysis of the metabolome has enabled rapid progress towards an in-depth understanding of the interaction between pathogen and host-cell metabolism and therefore disease pathogenesis. Furthermore, when applied to patient plasma or serum, metabolomics can profile metabolomic alterations in an infected patient which reflect systemic metabolic responses of diverse cell types in various organ systems affected by a specific pathogen, thereby identifying potential biomarkers of infection. Since metabolomics has the capability of identifying the entire collection of metabolites (e.g., lipids, carbohydrates, and amino acids) in a given sample, it is an emerging approach in clinical diagnostics.

**3. Immunometabolism**

The interplay between metabolism and the immune response is increasingly recognized, and distinct metabolic needs and demands across ages define responses to infection and vaccination [35]. Immunometabolism is a rapidly evolving field of immunology, and metabolites produced by leukocytes serve as potent immune signaling molecules, both during primary and trained immunity [36]. Trained immunity, the phenomenon of antigen-agnostic memory responses by innate immune cells subjected to a variety of environmental threats, is an evolutionary process mediated by epigenetic modulations [37]. Global or targeted metabolomic analysis of pre-defined immune cell populations can lead to novel discoveries of metabolic pathways or molecules that serve as epigenetic or transcriptional regulators. For example, mammalian target of rapamycin (mTOR) signaling, primarily through activating the transcription factor hypoxia-inducible factor-1 (HIF-1$\alpha$), increases aerobic glycolysis and regulates the differentiation of CD4+ T cells, favoring differentiation into Th17 cells versus Tregs and production of pro-inflammatory cytokines in response to T cell receptor activation [38]. In contrast, adenosine monophosphate-activated protein kinase (AMPK), an energy sensor kinase, serves as an immunometabolic checkpoint in T cell development and effector responses, as well as memory T-cell differentiation by regulating the metabolic switch from aerobic glycolysis to oxidative phosphorylation of lipids [39]. Lipids are used by immune cells for the synthesis of cell membranes, or posttranslational modifications of proteins [40] and lipid metabolism is of particular interest as it is implicated in epigenetic reprogramming of the immune response [41]. Integration of metabolomics with other 'omic readouts can lead to the discovery of mechanistic immunity networks and the validation of immune signatures [34]. Understanding the metabolic state and immunometabolic reprogramming of innate and adaptive immune cell populations during their exposure to pathogens or pathogen-associated molecular patterns (PAMPs) may inform discovery and development of target-based therapeutics and vaccines.

**3. Impact of infection on host metabolic signatures**

- During infection, several metabolic changes occur with reciprocal effects between the pathogen and the host. Metabolic adaptations occurring in eukaryotic hosts upon acute infection by bacterial or viral pathogens are complex, as the pathogen tries to profit from host nutrients and other metabolites to satisfy its bioenergetic and biosynthetic requirements while the host response is aimed at elimination of the invading pathogen [42]. Most bacteria and viruses enhance specific anabolic

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pathways in the host and are highly dependent on these alterations, suggesting that understanding and describing the pathogen-induced metabolic alterations in the infected host cell is a promising strategy for diagnostic, prognostic and therapeutic applications[43]. However, mapping accurate metabolic signatures produced by pathogens in infected hosts is challenging. Most of the studies so far provide only little information on metabolic changes triggered, due to the variable nature of metabolic responses to microbial pathogens. Select studies investigating metabolic signatures of infection in the human host are listed in Table 1.

**Table 1.** Metabolomic studies of selected infections in humans

| Infectious microbe | Examples of host metabolic pathways/ signatures perturbed | References |
|---|---|---|
| Chikungunya | Azelaic acid, Mandelic acid, Methylguanidine, D-Maltose, Ethanol, 2-Hydroxycaproic acid, Gluconolactone, Carnitine, Galactitol, Glycine, serine and threonine metabolism, Galactose metabolism and Citrate cycle | [44] |
| SARS-CoV-2 | Tryptophan, kynurenine, free fatty, sphingomyelin, diacylglycerols, glucose, glucuronate, bilirubin, bile acids, malic acid, glycerol 3-phosphate, triglycerides, phosphatidylcholines | [18, 45-49] |
| Dengue | Bile acids, purines, acylcarnitines, phospholipids, aminoacids, chenodeoxyglycocholic acid, uric acid, glycine, serine, threonine, galactose and pyrimidine, kynurenine, serotonin, starch and sucrose, glyoxylate and dicarboxylate, pentose phosphate pathway, propanoate | [44, 50-52] |
| Influenza A | Purine, lipid, glutathione, glucose, amino acids and ketone bodies, N-acetylglucosamine(O-GlcNAc), cAMP | [53-56] |
| Tuberculosis | Tryptophan, glutamate, sulfoxymethionine, aspartate, glutamine, methionine, asparagine, fatty acid metabolism, protein digestion pathway, lysosome pathway, sphingosine-1-phosphate, sphingolipid, amino-acyl tRNA | [17, 57-69] |

Common metabolic reactions are part of the host cell defense upon bacterial infection [70] to prevent access to nutrients by the respective pathogen [71]. Specific host responses triggered by bacterial pathogens include: (a) induction of host cell reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI), (b) enhancement of glucose uptake which stimulates the host cell's anabolic activity, (c) a switch to enhanced glutaminolysis and citrate lyase reaction enhancing fatty acid/lipid biosynthesis, and (d) an overall increase in lipid metabolism, especially biosynthesis of steroids and eicosanoids [72, 73]

Metabolic signature identification in pulmonary tuberculosis (TB) employing plasma high-resolution metabolomics (HRM) revealed that tryptophan metabolism is highly regulated throughout the spectrum of TB infection and disease, and is characterized by increased catabolism of tryptophan to kynurenine, which occurs not only in persons with TB disease but also in asymptomatic persons with latent TB infection (LTBI) [57]. Increased tryptophan catabolism may enable survival of *M. tuberculosis* (Mtb) at the site of infection by inducing immune tolerance and

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bacterial persistence and could also protect the host from excessive inflammation. Untargeted metabolite profiling of Mtb growing on $^{13}$C-labeled carbon substrates also revealed that Mtb could catabolize multiple carbon sources (dextrose, acetate, and glycerol) simultaneously to achieve augment growth [58]. Another TB study also demonstrated that levels of serum metabolites could differentiate adults with active pulmonary TB from both adults with LTBI and adults with no TB infection. Metabolic profiles revealed higher serum levels of glutamate, sulfoxy-methionine, and aspartate and lower serum levels of glutamine, methionine, and asparagine in active TB patients than in LTBI subjects or healthy controls [59].

Metabolomics can also be used as an effective method for identifying individuals at high risk of progression to active TB disease as ~10% of individuals latently infected with Mtb will progress to active disease at some point in their lives. Several key immunometabolic pathways associated with TB disease progression discriminate active TB from latent TB [65]. Fatty-acid metabolic networks are critical in TB progression as Mtb favors fatty acids as its cellular nutrient source and hosts several genes dedicated to fatty acid metabolism, higher than any other microorganism [66, 67]. The sphingolipid metabolic pathway is another established mediator of the host response to TB [69]. Sphingolipid metabolism is a key building block pathway of cell membranes which also play important roles in immune signaling and represent major constituents of the mucus secreted by lung alveolar epithelial cells [17]. Lastly, the amino-acyl tRNA pathway is associated with progression of TB infection to disease, with progressors showing a significant decrease of amino-acid levels compared to controls.

Viruses depend on the host cell to obtain the macromolecules and biosynthesis machinery for their replication and shape host-cell metabolism according to their specific needs [74]. Reprogramming of host metabolism supports viral pathogenesis by fueling viral proliferation and survival, enhancing access to free amino acids, fatty acids and host-derived lipid membranes, and eventually augmenting intercellular transmission and evading the host's immune system [75]. Some of the major cellular metabolic pathways, including glycolysis, fatty acid synthesis and glutaminolysis, are significantly altered by multiple virus families such as HCMV, HCV, HSV-1, and Poliovirus [74]. Serum metabolomics of chikungunya and/or dengue (co)infection revealed that glycine, serine, threonine, galactose and pyrimidine metabolisms are the most perturbed pathways in both single and co-infection conditions [44]. Tryptophan metabolites serotonin and kynurenin are differently enriched in patients with dengue hemorrhagic fever (DHF) suggesting that these metabolites can be used in combination with IFN-$\gamma$ as accurate metrics for early prognosis of DHF [51]. Analysis of Influenza A virus-infected cells reveals alterations in several metabolites of the purine, lipid and glutathione pathways, resulting in acceleration of viral replication [20]. Interestingly, the interaction between glucose metabolism and the inflammatory cytokine network might trigger a systemic inflammatory response in the host. Metabolomics of peripheral blood mononuclear cells (PBMCs) challenged with Influenza A virus (IAV) or H1N1 showed that increase in glucose metabolism promotes viral replication and cytokine production [55]. Plasma metabolomics of study participants with H1N1 influenza A pneumonia or bacterial community-acquired pneumonia (CAP) demonstrated changes in the concentration of some common and specific metabolites in H1N1 pneumonia compared with CAP with positive bacterial cultures. These studies demonstrated a decrease in citrate, fumarate, alanine and tyrosine and an increase in carnitine, glycine and acetoacetate in H1N1 patients [54].

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Detecting changes in the metabolome of infected patients might guide diagnosis and prognosis. In the context of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic, advances in metabolomics can shed light on the pathogenesis of coronavirus disease 2019 (COVID-19) that could inform development of therapeutics for these patients. Patients with COVID-19 exhibit changes in serum tryptophan metabolism and increased circulating levels of glucose and free fatty acids, consistent with altered carbon homeostasis, compared with SARS-CoV-2–negative controls. Interestingly, these findings correlate with clinical laboratory markers of inflammation (interleukin-6 (IL-6) and C-reactive protein) and renal function (i.e., blood urea nitrogen) [46]. Plasma lipid alterations (i.e. enhanced levels of sphingomyelins (SMs) and monosialodihexosylgangliosides (GM3s), and reduced diacylglycerols (DAGs)) associated with COVID-19 detected by targeted and untargeted tandem mass spectrometry analysis of the plasma lipidome and metabolome in mild, moderate, and severe COVID-19 and unaffected controls, suggest that SARS-CoV-2 might take advantage of host-derived lipid membranes [47], as has been described for other coronaviruses [48]. Metabolic signatures may predict early detection of infected patients at risk for severe disease in advance of severe clinical manifestations. Proteomic and metabolomic profiling of sera from COVID-19 and control individuals demonstrated that elevation of glucose, glucuronate, bilirubin degradation products, and four bile acid derivatives potentially indicates compromised liver detoxification function in those with severe COVID[18]. Plasma metabolite and lipid alterations are more extensive in fatal COVID-19 cases than in patients with severe and mild symptoms [49]. Among the most differentially regulated metabolites, malic acid and glycerol 3-phosphate, which are metabolites from carbohydrates pathway, show the greatest reduction in patients who die, followed by dramatic reductions in severely or mildly symptomatic patients compared to healthy volunteers. Plasma lipidomic alterations relate to clinical symptoms of COVID-19; diglyceride (DG), free fatty acid (FAA), and triglyceride (TG) levels increase with disease deterioration while phosphatidylcholines (PC) levels decrease over the course of COVID-19 fatal illness [49].

## 4. Metabolic signatures of vaccine-induced responses

Application of metabolomics to vaccine research may identify metabolites that correlate with immunogenicity and allow for individualized vaccination regimens for different populations. Through the application of global molecular 'omic techniques, systems vaccinology has set out to provide new insights into distinct age- and sex- specific vaccine-induced responses [76-78]. The metabolome, reflecting both genetic and epigenetic influences, shifts upon immune activation and can, in turn, shape immune responses. Plasma metabolic components, including small molecules and lipids, have immunomodulatory effects that may impact vaccine immunogenicity and responses to infection.

Limited systems vaccinology studies have sought to identify critical metabolic pathways correlating to vaccine induced signatures of protection [13, 79, 80] (Table 2). Metabolomic studies of influenza immunization of antibiotic-treated study participants demonstrated significant changes in cofactor/vitamin-related metabolism at Day 7 postvaccination compared to baseline [79]. Similarly, dysbiosis of bile acid metabolism occurs with the administration of antibiotics after influenza vaccines and IgG1 response was associated with metabolic clusters in fatty acid metabolism. This demonstrates that the perturbation of the microbiome regulates the availability of critical metabolites altering the

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immune responses to vaccination [79]. Plasma metabolomic analysis of immune responses to herpes zoster (shingles) vaccines demonstrates a strong association of transcriptomic pathways with multiple metabolic pathways, including lipid (e.g., glycerophospholipid, glycosphingolipid, and linoleate metabolism) and amino acid pathways (methionine and cysteine) at Day 3 postvaccination [13]. These metabolic pathways are also strongly associated with genes expressing MHC-TLR7/8 cluster, antigen presentation, dendritic cell (DC) activation, and B cell signatures [13]. Although no association between transcriptomics and metabolomics at Day 7 postvaccination and negative association was found on purine and lysine metabolism, the kinetics of vaccine-induced metabolic shifts may be dependent on gene expression [13]. In a phase III multicenter clinical trial, Hantavax vaccination (Hantavirus vaccine) reveals dose-dependent upregulation in folate biosynthesis, nicotinate, and nicotinamide, arachidonic acid, thiamine, and pyrimidine in the high responder group [80]. Different levels of response among participants signify robust differential metabolic phenomena. Antibody responses of high-responders strongly correlated with the metabolites cholesteryl nitrolinoleate, octanoyl-carnitine, tyrosine, ubiquinone-9, and benzoate, while chenodeoxycholic and methyl palmitate are upregulated in non- and low-responders. Such systems vaccinology studies have identified metabolic pathways that may be important to vaccine immunogenicity and may ultimately inform future vaccine design.

**Table 2.** Metabolomic studies of human vaccine responses

| Microbial Target | Vaccine formulation studied | Examples of metabolic pathways perturbed | References |
|---|---|---|---|
| *Francisella tularensis* | *F. tularensis* (LVS-DynPort Vaccine) | 2-oxocarboxylic acid, purine, asparagine, glycolysis, TCA-cycle, pyruvate | [81] |
| Hantavirus | Hantavax (GreenCross) | Arginine, phenylalanine, cholesteryl nitrolinoate, octanoylcarnitine, chenodeoxycholic acid, methyl palmitate, tyrosine, N-stearoyl tyrosine, 16-hydroxyplamitate, ubiquinone-9, benzoate, indole 3-acetaldehyde, arachidonic acid | [80] |
| Influenza | Fluzone (2014-2015, 2015-2016)*co-administered with antibiotics | Tryptophan metabolism, primary and secondary bile acids | [79] |
| Shingles | Zostavax | TCA cycle, glycolysis, gluconeogenesis, propanoate, ascorbate, aldarate, tryptophan, sterol, inositol phosphate | [13] |
| Small Pox | DryVax or ACAM 2000 | Creatinine, lysine, propylene glycol, lactate/threonine, alanine, lactate, 2-aminobutyrate, choline, glutamate, glutamine, methionine, creatinine, fructose, histidine, serine | [82] |
| Tuberculosis | BCG (Connaught strain) | Glucose processing metabolites, de novo purine synthesis, 1,5-anhydroglucitol, alpha-ketobutyrate, N6-carbomoyltheronyladenosine, methylguanine | [83] |

Metabolomics is also being applied to study vaccine safety. A human plasma metabolomics study of two hundred individuals pre- and post- small pox immunization discovered novel biomarkers for an adverse event following immunization (AEFI) reaction including redness at the local

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site of vaccination or severe allergic reaction[84]. This study reported that individuals who experienced clinically verified myocarditis or asymptomatic elevation of troponins were metabolically distinct compared to controls or those who only experienced systemic symptoms[84]. Creatinine, fructose, phenylalanine, histidine, and serine metabolites were decreased in those with an identified adverse event, implying certain vaccine adverse events may be metabolically-mediated[84]. Metabolomics, coupled with machine learning approaches, may predict the effects of vaccines and potentially help avoid serious adverse events following immunization, thereby improving the risk-benefit ratio of immunization. In the race of a SARS-CoV-2 vaccine, metabolomic approaches may help identify vaccine candidates that demonstrate relatively low reactogenicity and high protection. Leveraging systems biology approaches, including metabolomics, may further enhance benefit to risk ratios and the quality of immunization programs a topic of critical importance in an era of frequent vaccine hesitancy.

## 5. Integrative metabolomics- challenges and emerging horizon

The application of metabolomics for precision medicine is dependent on the ability to categorize e.g. infectious disease states or vaccine immunogenicity based on reproducible metabolic signatures that reflect specific discernible phenotypes, and can be used as a consistent readout [7]. For all its many strengths, the field of metabolomics is lagging behind other omics fields in terms of standardization of protocols [31] and routine usage of repositories for mass spectrometry data [85]. Close coordination between researchers, clinicians, and biomedical centers, will be necessary to identify reproducible and generalizable metabolic signatures in order to realize the full potential of metabolomics.

A potential obstacle to broader application of metabolomics for precision medicine has been the prohibitive cost of clinical metabolite profiling. Currently the typical cost of metabolomics is hundreds of dollars per sample, such that broad application of the technology would be unaffordable, limiting metabolomics-based precision medicine to well-funded clinical trials and perhaps the most privileged patients. To overcome this limitation, technologies such as NMR, which is more affordable than mass spectrometry [86], could be employed, acknowledging limitations in their detection abilities. Current efforts to reduce the cost of mass spectrometry-based methods by several companies might result in a significant reduction in the metabolite profiling costs over the next few years.

As the metabolomics research community finds ways to overcome the current limitations of this powerful technology, this will pave the way be personalized metabolic phenotyping [87, 88], which holds great promise in early diagnosis [89], informed treatment choices [90], and accurate prediction of response to therapy [91]. Of note, metabolomics data has advantages as compared to other screening methods as it directly measures drug metabolism, inflammation markers, and other ever-changing indicators of the patient's disease status. Such metabolomics data is most useful when integrated with clinical data (such as race, age, gender, clinical history of the patient, etc.) and molecular data (genomic, transcriptomic, and proteomic data) to inform a comprehensive report of the patient's health status.

Finally, robust structures and incentives for data sharing will be important to the success of the metabolomics field. Extensive studies by investigators in the fields of infectious diseases, vaccinology and metabolomics, coupled with data-sharing, such as that via the Human Immunology Project

Consortium ImmPort database[92, 93] (www.immport.org) will enable discovery and validation of signatures important to infection and immunization. Eventually such knowledge, when integrated with clinical information and validated in clinical contexts, may provide healthcare providers and vaccinologist the tools to leverage metabolic signatures for infectious disease diagnosis and vaccine development. Given its power and growing evidence that metabolic pathways are relevant to immune responses to infection and vaccination, we predict that collaborative data verification and integration efforts will eventually enable use of metabolic signatures to inform clinical decision making.

## 6. Conclusions

As the field of metabolomics evolves in accessibility, usability, and sophistication, metabolomic data will increasingly be integrated with clinical and other systems biology data to gain deep insights into the biology of health and disease. Given emerging evidence that metabolism plays key roles in infection and vaccine responses, a growing number of metabolites will likely emerge as targets for the development of biomarkers, prognostic factors, therapeutic targets and preventative modalities.

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**Conflicts of Interest:** Ofer Levy is a named inventor on several Boston Children's Hospital patent applications relating to human *in vitro* assays systems and vaccine adjuvants. The other authors declare no competing financial interests.

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