Metabolomics of urinary tract infection : a multiplatform approach

Pacchiarotta, T.

Chapter 1

Metabolomics investigation of human infections

Tiziana Pacchiarotta, André M. Deelder, Oleg A. Mayboroda

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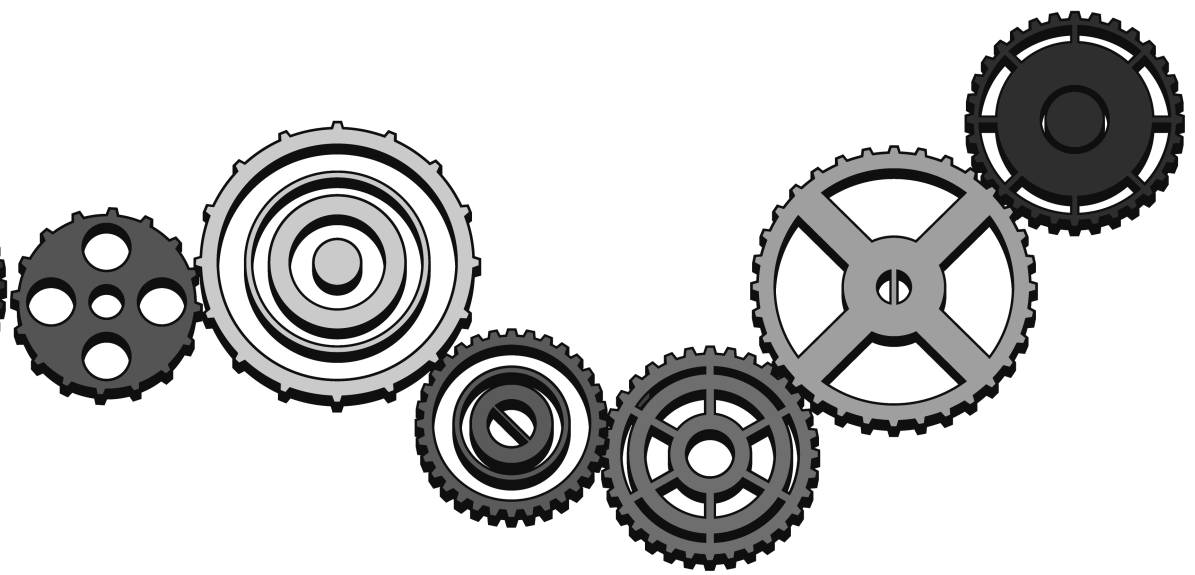

ABSTRACT

Metabolomics has a special place among other 'omics' disciplines (genomics, transcriptomics and proteomics) as it describes the most dynamic level of biological regulation and, as such, provides the most direct reflection of a physiological status of an organism. Quick development of the analytical technologies in the first place –MS and NMR– has enabled the metabolomics analysis of such complex biological phenomena as host-pathogen interactions in the development of infection. In this review, an overview of the metabolomics studies of the infectious diseases carried out on human material is provided. The relevant papers on the metabolomics of human infectious diseases are comprehensively summarized in a table, including, for example, information on the study design, number of subjects, employed technology and metabolic discriminator. Future considerations, such as importance of the time-resolved study designs and the embedment of metabolomics in the large-scale epidemiological studies are discussed.

BACKGROUND

Recent advances in technology have strongly stimulated the development of the exploratory study models known otherwise as 'omics', for example, genomics, proteomics and metabolomics. The latter one refers to a study of the metabolome –the 'complete' set of the metabolites (small-molecules such as metabolic intermediates, amino acids or hormones) to be found within a biological sample [1, 2]. As the metabolome provides the most direct reflection of a physiological status or an organism, metabolomics is increasingly integrated in clinical studies as an essential phenotypic measure. A good overview of the technological basis and current achievements of metabolomics can be found in a number of excellent reviews that have been published over the last decade [3, 4]. Here, however, we will concentrate only on the progress in the field of metabolomics related to studies on of infectious diseases.

There are several reasons why metabolomics of infectious diseases requires special attention. First, every infectious disease is a process of host-pathogen interaction; thus with regard to metabolomics, the metabolic signature of an infected host represents the reciprocal actions of the host and the pathogen or their metabolic cross-talk [5]. Second, although the risk of exposure to a pathogen and the environment of a host play the most important part, genetic susceptibility of the host to an infection and consequently the host's metabolic individuality should not be underestimated. Finally the enormous diversity of pathogens should be mentioned. The evolutionary distance between pathogens is considerable; their 'strategies' for invasion of the host and evasion of the host's immune system differ extremely, which makes any straightforward generalization questionable.

Such a diversity complicates a review as well, since our perception of the progress in the field will be affected by the perspective taken. One could, for example, build an overview grouping the available material as traditional, emerging or re-emerging infections, or use a biological classification of the pathogens: bacterial pathogens, viruses, protozoa, helminths. Alternatively, one could consider a problem from the perspective of the type of the infection - acute or chronic. Assuming that the 'metabolic costs' and mechanisms mobilized by the host during the acute response are different from those used in the chronic phase, an overview of the metabolomics of human infections from a such point of view appears to be the most logical one.

METABOLOMICS OF THE INFECTIONS IN THE ACUTE PHASE

Every pathogen has to survive the acute-phase response of the host, but the development of the acute phase into a life-threatening condition happens not so often. Many pathogens are capable of subverting the initial reaction of the host and progress to a chronic phase. Consequently, here we try to address only those acute infections that are known as distinct clinical entities, such as, for example, urinary tract infection (UTI), which is indeed the most common bacterial infection leading to considerable health care expenditures. In fact the term 'urinary tract infection' encompasses a variety of clinical syndromes with the common denominator of a positive urine culture (i.e., significant bacteriuria > 10^3 CFU/ml) [6]. However, the presence of bacteria in urine is essential but not sufficient for the diagnosis of UTI and the correct assessment of the disease relies on a coordinated effort of clinicians and microbiologists. Within the limited number of metabolomics studies that have been performed on clinical material, UTI appears to be the most common topic.

The technical aspects, namely the assessment of the feasibility of analytical methods to

be applied, play an important role in those studies, regardless of whether an exotic technology is introduced [7] or a standard metabolomics technology, for example LC-MS, is used. A recent study of Lv *et al.* represents a good example of this technology-driven approach. The study is dedicated to the development of an LC-MS based method for metabolic profiling of UTI samples [8]: it includes optimization of the protocol, testing of columns with different particle sizes and both positive and negative ionization modes. The technology validation was followed by the analysis of a limited selection of samples. The authors thus identified a number of metabolites being specific for UTI (Table 1). However, it might be argued that far reaching biological conclusions, based on analysis of only a few samples, may need a critical re-evaluation. The work of Pacchiarotta *et al.* is another example of the use of the LC-MS technology for studying metabolomics of UTI [9]. Taking advantage of a prospective observational multicenter cohort study, the authors have selected a group of patients with culture-confirmed febrile Escherichia coli UTI. The study design included the control samples and samples of UTI patients collected at baseline (t=0) as well as patient samples collected after the antibiotic treatment (t=30). Thus, the authors had the possibility to not only compare UTI patients with symptom-free controls but also to observe how the metabolic phenotype of the patients reverts after the treatment. In doing so, they identified unique *O*-glycosylated fragments of the human fibrinogen alpha 1-chain and showed that presence of those fragments in urine strongly correlated with UTI symptoms.

Slupsky *et al.* explored another clinically important infection, namely acute pneumococcal pneumonia [10]. Pneumonia, like UTI, is a complex clinical entity, where an infection of the lower respiratory tract caused by bacteria or viruses is the common feature. The pneumococcal pneumonia caused by *Streptococcus pneumonia* is the main community-acquired form of the disease. To dissect the metabolic signature ('metabotype' according to authors' terminology) specific for S. pneumonia infection, a combination of targeted quantitative NMR profiling (61 metabolites) and a rather sophisticated study design was used. Anticipating that an acute infection represents a situation of metabolic stress for the host, the authors took a number of steps to ensure the specificity of their findings: the selection of patients included several additional 'control' groups of patients such as patients with non-infectious metabolic stress (cardio-vascular failure), patients with lung infections caused by other pathogens, fasting individuals and so on. Furthermore, a small longitudinal study on a sub-selection of eight samples was undertaken to monitor the evolution the 'metabotypes' of the patients. The approach used by the authors in the study design should serve as an example for future studies on metabolomics of infectious diseases. Still, one might ask whether an exploratory analysis of the entire NMR spectra could reveal more interesting compounds than those which were included in the list.

There are number of clinically relevant pathogens which present a threat to the human host during the acute phase of the infection. At first glance, it appears that many of those have been well studied and some (e.g., *Salmonella sp.*) have even been 'developed' into the standard model systems. However, the bulk of the publications address the metabolism of the pathogens in vitro or in animal models and the real clinical metabolomics studies of such important pathogens as *Vibrio cholerae* or even the otherwise well studied *Salmonella* are still awaiting their time.

Acute viral infections, such as influenza, represent a serious threat to the global health system. The previous pandemics (1918, 1957 and 1968) have clearly demonstrated the scope of the problem [11]. The 'efficiency' of influenza virus is based on the genetic re-assortment

and the effect of antigenic drift –a gradual change of the viral antigenic pattern due to frequent mutations [12]. Thus, even if a human host will acquire immunity to the invading strain, every new strain, originated as a result of the antigenic drift, can evade the host immune system. The value of the metabolomics studies in global control and management of influenza is far from being self-evident, which probably explains the lack of such reports in literature. There are, however, some publications addressing the impact of the virus on the metabolism of the hosts' cells. For example, Lin *et al.* have explored the metabolic pattern of human cell lines infected with influenza A virus [13]. They indicated that the metabolic signature might be associated with differentiated stages of the cells, probably due to fatty acids and cholesterol biosynthesis. Another possible niche for metabolomics study in a context of influenza infection could be an exploration of the 'collateral damage' effects like influenza-associated encephalopathy characterized by abrupt onsets of unconsciousness. The last case was addressed by Kavashima *et al.* who have studied CSF samples from six children affected by influenza-associated encephalopathy [14]. Using a high-field mass spectrometry (7 Tesla APEX III FTMS, Bruker Daltonics) authors have identified a number of signals specific for influenza-associated encephalopathy. However, neither statistical validation of the results, nor structural assignment of the signals, was presented.

METABOLOMICS OF THE INFECTIONS IN THE CHRONIC PHASE

If the question 'what do viruses and helminths have in common?' will ever be asked, a possible answer could be that these groups of organisms are 'masters of subversion'. To formulate it in more formal language, many viruses and helminths have the exceptional ability to develop long-lasting infections and coexist with the host for years or even decades. Hepatitis C virus (HCV) is a good example: in early infection the virus presents little to no damage to the host, however in the chronic infection it leads to progressive loss of liver function in 70% of patients [15]. Liver biopsy is still considered an essential element of HCV management and, thus, it comes as no surprise that one of the first applications of a metabolomics approach to this infection was an attempt to find an alternative way for the estimation of HCV-related morbidity. Zhang *et al.* have used targeted profiling of amino acids to reassess an old association between liver fibrosis and Fisher's ratio: the molar ratio of branched amino acids to aromatic ones. [16, 17]. A chronic Hepatitis B infection also results in the losses of liver function and an increase in the risk of hepatocellular carcinoma. A strong correlation between this morbidity and the levels of free amino acids in serum of HVB infected patients has been shown by Yu *et al.* [18].

An overview of the work carried out on HIV would require a separate review. There is a large body of literature dedicated to the studies of antiviral drugs and the metabolism of human CD4+ T-cells infected with the virus. Furthermore, an in-depth analysis of the metabolomics of HIV should discuss a fraction of the literature dedicated to the fundamentals of the human metabolism in the context of HIV infection and effects of the nutritional status of the host. These studies seldom use the 'metabolomics rhetoric' but they are essential for understanding the long-term effects of the infection. 'Classical' large-scale metabolomics studies on HIV are still lacking. However an original approach to the problem was taken by Ghannoum *et al.* who have evaluated oral wash samples as potential material for HIV diagnostics and monitoring of antiviral treatment [19]. Using a combination of

LC- and GC-MS, the authors have generated an overview of the metabolic composition of the oral cavity. The identity of all discussed metabolites has been verified by a custom built library of analytical standards. Next to the comparison between a control group and HIV patients a table summarizing the metabolic differences amongst HIV patients (naïve and exposed to the antiretroviral therapy) was presented. However, a closer examination of the reported p-values shows that the differences within the HIV group (naïve vs. antiretroviral therapy patients) are apparently more significant than between HIV and control groups. An interesting fact, which unfortunately was not discussed by the authors. While the biological system assumptions of the authors might need an independent validation on a larger set of samples, the idea to use the metabolome of the oral cavity for monitoring of the status of a viral infection opens many interesting possibilities. Oral wash has indeed a number of advantages: it is safer to handle than blood, it allows noninvasive sampling, the volume of the sample can be controlled and finally it is always available, which makes it an almost ideal sample for the 'time-resolved' studies [19].

A long-lasting coexistence with the host is typical for the survival of many parasitic helminths species. Some of these helminths, for example trematodes of the genus Schistosoma, can survive for decades. Schistosomiasis is after malaria the second most socio-economically devastating parasitic infection. According to the World Health Organization (WHO) there are approximately 200 million people chronically infected and 600 million at risk of the infection (www.who.int). Unlike malaria, where a large-scale clinical metabolomics study is still lacking, schistosomiasis has already attracted the attention of the metabolomics community. Two large NMR studies have been published within 4 years [20, 21]. Both studies were based on material collected in endemic areas: Ivory Coast [20] and in Uganda [21]. Despite many factors (geographical location, nutritional habits, co-infections) that make a direct comparison of the two studies difficult, both groups have reported that the age of the participants is a dominant source of the variance the data overshadowing all other factors including infection status and parasitic load. Anticipating this effect, Balog *et al*. have analysed their cohort by splitting it in two age-matched sub-groups; the first one included participants of 7-15 years old and the second of 20-40 years old. The list of the metabolic discriminators presented by the authors includes no surprising entries, but the data overlap significantly with results obtained on animal models [20, 22].

It is evident that viruses and helminths are not the only organisms causing chronic infections; bacterial pathogens, such as mycobacteria, can also be very efficient in subversion of the host defence and in long-time survival. The best example is *Mycobacterium tuberculosis* - the causative agent of the one of the most widespread of all human infectious diseases - tuberculosis (TB). The impact of the TB on the human population is so profound that the WHO has declared the state of a Global Emergency in 1993. With the development of multi-drug resistant tuberculosis, the decision of the WHO can hardly be considered as an overreaction. The value of the metabolomics for a systemic approach to the TB problem has been recognised already for some time and has even been addressed in several reviews [23, 24]. However, no metabolomics study on human material has been published so far and a study on a mouse model remains our closest approximation [25]. Leprosy, caused by Mycobacterium leprae, is another important mycobacterial infection. In comparison to TB, leprosy might appear to be a relatively minor problem but more than 200000 new cases worldwide were reported in 2010 alone [26] and this disease still represents a peculiar case when physical disability often enhanced by the social stigma associated with it [27]. The

work by Al-Moubarak *et al*. on metabolomics of *Mycobacterium leprae* [28] is to the best of our knowledge the only metabolomics study of a Mycobacterium infection performed on human material. The authors employed UPLC-MS for their study and used serum samples from a sample bank of an on-going project on the molecular epidemiology of leprosy. This technologically solid study has nevertheless one small weakness with respect to the sample selection. By comparing patients with high and low bacterial indices (BI), and by using a random sample selection from the database they ended up with two very unbalanced groups. The group of high BI consisted of only males (only one female) while the same time group of low BI was mixed. Thus, it remains an open question to what extent the differences reported by the authors can be attributed to the differences in the infectious status.

FUTURE PERSPECTIVES

The current overview covers only a fraction of the literature on the metabolomics of infectious diseases, namely studies performed on human material. At a first glance, the progress in this field might appear insignificant in comparison to such areas as metabolic syndrome, cardiovascular disorders or cancer [29-31]; only a limited number of studies have appeared and the data on some common infectious diseases, such as TB or malaria remain unavailable. Furthermore, the sets of specific metabolites observed often consist of a selection of the usual suspects (carboxylic acids, amino acids, sugars; for more details see Table 1) leading to the quite common conclusion about the involvement of the energy metabolism in the host reaction to an infection.

On the other hand, the link between the regulation of the host energy metabolism and the response to an infection has been known for decades and one might argue that the ability of a new technology to expose this well-established physiological phenomenon should be considered as a positive fact which provides a solid foundation for future investigations [32-34]. With regard to the usual suspects, one could emphasize the fact that, in general, not one or two specific metabolites but a specific panel of metabolites or even the metabolic pattern/profile of an organism as a whole should serve as a basis for clinically relevant conclusions. However, to identify those metabolic patterns, to reveal their specificity and to show their feasibility for clinical research, the static case-control study design will have to be substituted by a longitudinal one or any other type of design where time-dependent phenomena can be explored. So far, only a few studies (Slupsky *et al.* [10], Pacchiarotta *et al.* [9] –bacterial pathogens– and Balog *et al.* [21] –parasitic infection) have employed such designs.

Although, metabolomics of the infectious diseases is still in its early days, we are confident in its future. In a way, it is, probably, the most systemic part of the systems biology: it studies a unique phenomenon of the interaction of a pathogenic organism with human super-organism [35]. At the moment, it is difficult to foresee the way this field will evolve. Yet we might speculate that in the case of acute infections, a practical approach will not be the development of the diagnostics, which is usually established in clinical laboratories, but, for instance, the development of the analytically based scoring systems for the scaling of morbidity, or estimation of the response to treatment. The latter aspect is becoming increasingly important as the list of antibiotic-resistant strains is becoming longer and longer. With regard to chronic infections, an exploration of the metabolic trajectories of the infections unfolding in time is probably the most logical way to achieve the practical results. Finally, to address difficult cases - TB, HVC or HIV - the metabolomics should be integrated

in the large-scale epidemiological projects as a standard phenotypic measure.

Table 1. Overview of the works available on human material divided by acute and chronic infections.

Pathogen	Biofluids	Technique	Metabolite discriminator	Number of subjects	Study approach *	Publication year [Ref. Number]
Streptococcus Penumoniae	Urine	NMR	TCA cycle intermediates, branched amino acids, creatinine, taurine, myo-inositol	641	1, 2	2009 [10]
E. Coli	Urine	CE-MS	Phenylalanine, glutamic acid	18	1	2008 [7]
E. Coli, Gram positive	Urine	UFLC-MS	TCA cycle, terpenoid backbone biosynthesis, amino sugars and nucleotide sugars metabolism, arachidonic acid and steroid hormone biosynthesis	17	1	2011 [8]
E. Coli	Urine	UPLC-MS	C-terminal glycopeptide of the fibrinogen alpha chain 1	117	1, 2	2012 [9]
Klebisella pmeumoniae	Urine/Cell media	NMR	Glycerol metabolism	614	1	2006 [36]
E. coli, K. pneumoniae, P. aeruginiosa, Pr. mirabilis	Urine/Cell media	NMR	Lactate metabolism, methionine metabolism, nicotinic acid	617	1	2009 [37]
E. coli, K. pneumoniae, P. aeruginiosa, Pr. mirabilis	Urine	SELDI-ToF, NMR	N/A	80	1	2003 [38]
Plasmodium falciparum	Urine	NMR	Lipids, lactate	39	1	1988 [39]
Plasmodium falciparum	Red blood cells	NMR	Gamma aminobutyric acid (GABA), TCA intermediates, glutathione			2009 [40]
Influenza virus	CSF	Direct Infusion MS	N/A	6	1	2006 [14]
Hepatitis C virus	Urine	NMR	N/A	66	1	2010 [41]
Hepatitis C virus	Plasma	HPLC-MS	Branched and Aromatic Amino acids	53	1	2006 [17]
Hepatitis B virus	Serum	GC-MS	Branched and Aromatic Amino acids	47	1	2006 [18]
Hepatitis B virus	Plasma	GC/MS	Acetic acid, sorbitol, D-lactic acid, hexanoic acid, 1-nephtalenamine, butanoic acid, phosphoric acid, D-glucitol, glucose	40	1	2009 [42]
Hepatitis B virus	Serum	LC-MS	Lysophosphatidyl cholines, bile acids, hypoxanthine stearamide	59	1	2011 [43]
Hepatitis B virus	Plasma	UPLC-MS	Lysophosphatidyl cholines, primary fatty acid amides, comjugate bile acids	83	1, 2	2011 [44]
HIV virus	Oral wash samples	GC-MS, LC MS	Phenylalanine:tyrosine metabolism, aminosugars metabolism, purine metabolism, fructose mannose galactose, starch and sucrose metabolism, TCA cycle	24	1	2011 [19]
HIV virus	Plasma	HPLC-fluorescence	Phenylalanine/Tyrosine	107	2	2010 [45]
HIV virus	Plasma	NMR	Lipids, Glucose	63	1	2006 [46]
Mycobacterium Leprae	Serum	UPLC-MS	Polyunsaturated Fatty Acids, phospholipidis	23	1	2011 [28]
Schistosoma mansoni	Urine	NMR	TCA cycle, liver function, gut microflora	447	1, 2	2011 [21]
Schistoma mansoni	Urine	NMR	N/A		1	2007 [20]
Onchocerciasis volvulus	Serum	LC-MS	Fatty acids, sterol lipids	73	1	2010 [47]

*1 = case control study, 2 = longitudinal study

REFERENCES

[1] Oliver SG, Winson MK, Kell DB, Baganz F. Systematic functional analysis of the yeast genome. Trends in Biotechnology. 1998;16:373-8.

[2] Fiehn O. Metabolomics - the link between genotypes and phenotypes. Plant Molecular Biology. 2002;48:155-71.

[3] Kell DB. Metabolomics and systems biology: making sense of the soup. Current Opinion in Microbiology. 2004;7:296-307.

[4] Holmes E. The evolution of metabolic profiling in parasitology. Parasitology. 2010;137:1437-49.

[5] Kafsack BFC, Llinas M. Eating at the Table of Another: Metabolomics of Host-Parasite Interactions. Cell Host & Microbe. 2010;7:90-9.

[6] Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis. 2004;38:1150-8.

[7] Ramautar R, Mayboroda OA, Derks RJE, van Nieuwkoop C, van Dissel JT, Sornsen GW, et al. Capillary electrophoresis-time of flight-mass spectrometry using noncovalently bilayer-coated capillaries for the analysis of amino acids in human urine. Electrophoresis. 2008;29:2714-22.

[8] Lv H, Hung CS, Chaturvedi KS, Hooton TM, Henderson JP. Development of an integrated metabolomic profiling approach for infectious diseases research. Analyst. 2011.

[9] Pacchiarotta T, Hensbergen PJ, Wuhrer M, van Nieuwkoop C, Nevedomskaya E, Derks RJE, et al. Fibrinogen alpha chain O-glycopeptides as possible markers of urinary tract infection. Journal of Proteomics. 2012;75:1067-73.

[10] Slupsky CM, Rankin KN, Fu H, Chang D, Rowe BH, Charles PGP, et al. Pneumococcal Pneumonia: Potential for Diagnosis through a Urinary Metabolic Profile. Journal of Proteome Research. 2009;8:5550-8.

[11] Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of Influenza-A viruses. Microbiological Reviews. 1992;56:152-79.

[12] Carrat F, Flahault A. Influenza vaccine: The challenge of antigenic drift. Vaccine. 2007;25:6852-62.

[13] Lin S, Liu N, Yang Z, Song W, Wang P, Chen H, et al. GC/MS-based metabolomics reveals fatty acid biosynthesis and cholesterol metabolism in cell lines infected with influenza A virus. Talanta. 2010;83:262-8.

[14] Kawashima H, Oguchi M, Ioi H, Amaha M, Yamanaka G, Kashiwagi Y, et al. Primary biomarkers in cerebral spinal fluid obtained from patients with influenza-associated encephalopathy analyzed by metabolomics. International Journal of Neuroscience. 2006;116:927-36.

[15] Schuppan D, Krebs A, Bauer M, Hahn EG. Hepatitis C and liver fibrosis. Cell Death Differ. 2003;10:S59-S67.

[16] Fischer JE, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB. Effect of normalization of plasma amino-acids on hepatic encephalopathy in man. Surgery. 1976;80:77-91.

[17] Zhang QW, Takahashi M, Noguchi Y, Sugimoto T, Kimura T, Okumura A, et al. Plasma amino acid profiles applied for diagnosis of advanced liver fibrosis in patients with chronic hepatitis C infection. Hepatol Res. 2006;34:170-7.

[18] Yu K, Sheng GP, Sheng JF, Chen YM, Xu W, Liu XL, et al. A metabonomic investigation on the biochemical perturbation in liver failure patients caused by hepatitis b virus. Journal of Proteome Research. 2007;6:2413-9.

[19] Ghannoum Mahmoud A. MPK, Jurevic Richard J.,Retuerto Mauricio, Brown Robert E., Sikaroodi Masoumeh, Webster-Cyriaque Jennifer, Gillevet Patrick M. Metabolomics Reveals Differential Levels of Oral Metabolites in HIV-Infected Patients: Toward Novel Diagnostic Targets. OMICS: A Journal of Integrative Biology. 2011.

[20] Singer BH, Utzinger J, Ryff CD, Wang Y, Holmes E. Chapter 11 - Exploiting the Potential of Metabonomics in Large Population Studies: Three Venues. In: John CL, Jeremy KN, Elaine HolmesA2 - John C. Lindon JKN, Elaine H, editors. The Handbook of Metabonomics and Metabolomics. Amsterdam: Elsevier Science

B.V.; 2007. p. 289-325.

[21] Balog CIA, Meissner A, Goraler S, Bladergroen MR, Vennervald BJ, Mayboroda OA, et al. Metabonomic investigation of human Schistosoma mansoni infection. Mol Biosyst. 2011;7:1473-80.

[22] Wang YL, Utzinger J, Xiao SH, Xue J, Nicholson JK, Tanner M, et al. System level metabolic effects of a Schistosoma japonicum infection in the Syrian hamster. Mol Biochem Parasitol. 2006;146:1-9.

[23] Sintchenko V, Iredell JR, Gilbert GL. Opinion - Pathogen profiling for disease management and surveillance. Nat Rev Microbiol. 2007;5:464-70.

[24] Forst CV. Host-pathogen systems biology. Drug Discovery Today. 2006;11:220-7.

[25] Shin J-H, Yang J-Y, Jeon B-Y, Yoon YJ, Cho S-N, Kang Y-H, et al. ^{1}H NMR-based Metabolomic Profiling in Mice Infected with Mycobacterium tuberculosis. Journal of Proteome Research. 2011;10:2238-47.

[26] Weekly epidemiological record. 2011. p. 389-400.

[27] Rafferty J. Curing the stigma of leprosy. Lepr Rev. 2005;76:119-26.

[28] Al-Mubarak R, Vander Heiden J, Broeckling CD, Balagon M, Brennan PJ, Vissa VD. Serum metabolomics reveals higher levels of polyunsaturated Fatty acids in lepromatous leprosy: potential markers for susceptibility and pathogenesis. Plos Neglect Trop Dis. 2011;5:e1303.

[29] Kind T, Tolstikov V, Fiehn O, Weiss RH. A comprehensive urinary metabolomic approach for identifying kidney cancer. Analytical Biochemistry. 2007;363:185-95.

[30] Lawton KA, Berger A, Mitchell M, Milgram KE, Evans AM, Guo L, et al. Analysis of the adult human plasma metabolome. Pharmacogenomics. 2008;9:383-97.

[31] Griffin JL, Atherton H, Shockcor J, Atzori L. Metabolomics as a tool for cardiac research. Nat Rev Cardiol. 2011;8:630-43.

[32] White RH, Frayn KN, Little RA, Threlfall CJ, Stoner HB, Irving MH. Hormonal and metabolic resposnse to glucose-infusion in sepsis studied by the hyperglycemic glucose clamp technique. Journal of Parenteral and Enteral Nutrition. 1987;11:345-53.

[33] Shaw JHF, Klein S, Wolfe RR. Assesment of alanine, urea, and glucose interrelationships in normal subjects and in patients with sepsis with stable isotopic tracers. Surgery. 1985;97:557-68.

[34] Carpentier YA DD, Siderova VS, Richelle M. Exogenous lipids and hepatic function. In: Kinney JM TH, editor. In Organ Metabolism and Nutrition: Ideas for Future Critical Care. New York: Raven Press, Ltd; 1994. p. 349-67.

[35] Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. Nat Rev Microbiol. 2005;3:431-8.

[36] Gupta A, Dwivedi M, Gowda GAN, Mahdi AA, Jain A, Ayyagari A, et al. H-1 NMR spectroscopy in the diagnosis of Klebsiella pneumoniae-induced urinary tract infection. NMR Biomed. 2006;19:1055-61.