Metabolomics in lupus; opportunities and challenges

Sara Afshari1, Narges Kalhor1, Seyed Mojtaba Alavi1, Seyed Mohammad Hashem Montazeri1, Maryam Masoumi2

1Student Research Committee, School of Medicine, Qom University of Medical Sciences, Qom, Iran
2Clinical Research Development Center, Shahid Beheshti Hospital, Qom University of Medical Sciences, Qom, Iran

Abstract

Systemic lupus erythematosus (SLE) is characterized by an inappropriate autoimmune response to self-antigens. This disease is a heterogeneous autoimmune disease that shows variable clinical course. Metabolomics employs advanced analytical chemistry techniques to comprehensively measure many small molecule metabolites in biological cells and tissues. Metabolites are downstream of translation processes and are thought to be associated with disease phenotypes. This technology is recognized as a powerful tool with excellent potential for detecting prognostic and diagnostic biomarkers in rheumatic diseases. In this review, we summarized the recent available results of studies on metabolomics in lupus and the importance of metabolomics in the finding of diagnostic and prognostic biomarkers was investigated.

Key point

SLE is an autoimmune disease with various clinical presentation. Due to heterogeneous characteristics of this disease, its diagnosis is challenging. Metabolites are produced by cell metabolisms. Metabolite profiles can be used as a possible biomarkers for SLE detection and prognosis.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease that may involve several organs of the body and show different clinical courses (1).

The incidence of SLE in females is approximately nine times that of males. It is estimated that SLE costs approximately $13 billion annually for US health care systems, indicating that SLE is a big diagnostic and therapeutic challenge for nations.

The disease is notably more frequent among African, Asian, Hispanic and local American populations and these experience the highest mortality rate, too (2,3).

SLE is characterized by an irrelevant autoimmune reaction to self-antigens. Environmental factors in genetically susceptible individuals administrate to break down self-tolerance and activate innate immunity of cells and autoreactive lymphocytes against body cells (4, 5).

Due to the heterogeneous character of lupus, a broad spectrum of clinical manifestations exists. The first clinical manifestations of SLE may appear as (fatigue), myalgia, fever, anorexia and weight loss. SLE usually involves several organs and systems, such as skin, joints, kidneys, lungs, central nervous system and hematopoietic system. Actually, no two cases of lupus are exactly alike (6-8).

Metabolomics

On the molecular level, the human body is an exceptionally dynamic system, with lots of chemical and molecular responses taking place within millions of cells at any given moment. These biochemical reactions are liable for preserving cell activity and maintaining cell shape and cell contact with one another (10). Metabolomics utilizes advanced analytical chemistry techniques to comprehensively measure many small molecule metabolites in biological cells and tissues. This technology is recognized as a powerful tool with excellent potential for detecting prognostic and diagnostic biomarkers in rheumatic diseases.
chemistry techniques to degree a large number of small molecule metabolites in biological cells and tissues and refers back to the systematic analysis of metabolites (low molecular weight biochemical substances such as carbohydrates, amino acids, organic acids, nucleotides and lipids) in an organic sample (10,11).

Small molecule metabolite profiles are capable to be used as potential biomarkers for the detection, prognosis of disease, response to treatment and disease status.

In addition, some genetic disturbances are associated with many diseases but they account for a little portion of the disease risk. Due to the multifactorial nature of disease mechanisms, the excessive influence of the environment and the intricacy of gene-environment interactions and also the effect of post-translational changes (10-12).

**Approaches to the metabolomics study**
The strategies for conducting metabolic tests are classified in two directions: 1) Non-targeted analysis, and 2) Targeted analysis.

Non-targeted metabolomic investigations are used as a hypothesis-generating strategy and are defined by qualitative and quantitative measurement of enormous numbers of metabolites in the samples (12). In this way, it is possible to create metabolic profiles, detect and analyze metabolites or panels like lipids, including phospholipids, amino compounds and carbohydrates without focusing on a particular compound.

Targeted metabolism concerns hypothesis-driven experiments and is defined by gathering quantitative data on a predetermined set of metabolites with high profile of precision. In fact, targeted methods include multiplexed analysis of known metabolites. For example, targeted metabolism, the measurement of analyzes based on common biochemistry and/or previously well-established non-targeted studies, is selected (13, 14).

Employing efficient statistical analysis, shifts in metabolites can be traced to definitive pathways, allowing information to be obtained throughout the pathophysiological process of a specific disease (12,14,15).

**Techniques for studying metabolomics**
Metabolism is usually utilized in two different ways:
1. Proton (1 H) nuclear magnetic resonance (NMR) spectroscopy
2. Mass spectrometry (MS) coupled with gas or liquid phase chromatography (GC and LC, respectively).

The samples are usually separated into gas (GC) or liquid chromatography (LC) before performing MR and the compounds are characterized by mass and fragmentation patterns (16,17). In contrast, NMR spectroscopy measures all metabolites at once without the need for separation, but this method is less sensitive (18).

Mass spectrometry is often used for semi-targeted or untargeted metabolomics because it is more sensitive, more powerful and also can determine more molecules in a biological specimen (19).

NMR detects molecular properties by measuring the intrinsic magnetic substances of atomic nuclei. NMR is selective and non-destructive but of relatively low sensitivity (20).

**Metabolomics as a possible tool for biomarker studies in SLE**
Initial diagnosis and differentiation between early SLE and other rheumatic diseases are incredibly difficult within the early stages and sometimes the disease has not been diagnosed for many years.

Common methods of diagnosing and evaluating the disease include the employ of acute phase markers, such as erythrocyte sedimentation rate and C-reactive protein and anti-dsDNA antibodies have inadequate sensitivity and specification (21).

Metabolite profiles of small molecules can be used as possible biomarkers for the detection and prognosis of the disease. Metabolomic studies in SLE have examined metabolic changes in SLE pathology over the past decade.

Metabolomics studies in lupus have been developed to screen for fewer invasive bio-fluids, such as serum or urine, for separating metabolomics related to disease activity and response to therapy. These will assist in diagnosing, evaluation of disease activity and treatment choices (22,23).

**Serum and plasma metabolomes**
Liang et al in 2018 described the coagulation cascade relationship to the complement system in SLE. Using LC-MS (liquid chromatography coupled to MS), the serum metabolome of 24 SLE patients was compared to 24 healthy controls. In addition, the levels of ten coagulation factors, seven complements and three cytokines were measured in 112 SLE patients. In this study, clinical data were obtained from 2025 SLE patients.

An total of 195 significantly changes in metabolites were seen in SLE patients. The main metabolites that are differentially displayed in SLE patients contain tyramine, L-tryptophan valproic acid, 1-alpha-25-dihydroxyvitamin, linoleic, L-leucine, L- 2-phenylethylamine. In this study the metabolic pathways that were changed in SLE patients have been determined, which include steroid hormone biosynthesis, tryptophan metabolism and interestingly coagulation cascade pathway and complement system.

The findings indicated that the coagulation cascade and complement system had cooperation effects on the severity of SLE. This analysis shows relationships between the coagulation cascade components and the complement system (24).

Another mass spectroscopy-based metabolomics study (LC/MS and GC/MS) referred to SLE that compared the metabolites in SLE patients and healthy controls, which showed100 metabolites were outstandingly different in SLE. Much of the difference observed is related to...
energy metabolism. The most important of these energy production pathways include glycolysis, the Krebs cycle and beta-oxidation of lipids. These findings mention a severe reduction in ATP production. The study also found an increase in oxidants and a decline in most free long-chain fatty acids in SLE patients (25).

Recent studies showed some members of the acylcarnitine family have undergone major changes in SLE. This indicates the dysregulation of β oxidation processes in this disease. This study also suggested a moderate-to-strong relation between elected individual metabolic peaks and bacterial genera in the bowel (26).

Urine SLE metabolome

In a study, the larger sample size was used to analyze urine metabolome profiling of six of the most common immune-mediated inflammatory diseases (IMIDs) (rheumatoid arthritis, SLE, psoriatic arthritis, Crohn's disease, psoriasis and ulcerative colitis) by the use of NMR. Compared to healthy controls, patients with immune-mediated inflammatory diseases (especially SLE) had 28 important associations between urinary metabolite level and diagnosis of disease. Moreover, significant relationships of three significant metabolites with disease activity were found [PFDR (false-discovery rate) <0.05]. Some of the metabolite variations were prevalent across all or almost all diseases, thereby, they were considered as hub metabolites. Citrate had the strongest hub properties, which in most of IMIDs showed a definitely lower concentration in the urine. Compared to controls, there were another five hub metabolites, which were significantly associated with several IMIDs. Compared to healthy controls, alanine, N-acetyl amino acids (N-acetyl AAs), methyl succinate and trigonelline displayed lower accumulation in the urine of some different IMIDs. Additionally, in the urine of SLE patients, lower level of citrate had been determined compared to controls (27).

Network analysis revealed lots of similarity between the three major metabolic pathways. The citric acid cycle was the main pathway identified and citrate had a common relationship with IMID. The phenylalanine metabolism pathway was the second major metabolic pathway. An important role of glycine and serine metabolism pathway was determined in IMIDs analyses.

Further comparison between patients with SLE and others revealed 11 metabolites were very different. It can use for differential diagnosis in IMIDs (27, 28).

Fecal SLE metabolome

This is a new metabolic model for better SLE diagnosis. Feces is also an excellent biological fluid for new biomarkers because of its uncomplicated and noninvasive collection.

Ultra-high-performance liquid chromatography equipped with mass spectrometry studies conducted by Zhang et al in 2019 compared fecal metabolic profiles of SLE patients with healthy controls for detecting a potential biomarker for SLE. Around 23 important metabolites that include the amino acids, purine, lipids and vitamin B metabolisms were changed in the feces of SLE group, compared with healthy group. The most important pathways that changed were aminoacyl-tRNA (aa-tRNA) biosynthesis, nitrogen metabolism, thiamine metabolism, tryptophan metabolism and cyanoamine acid metabolism. Interestingly glucogenic amino acids, such as L-methionine, proline, and L-asparagine increased in the stool of SLE patients. Furthermore, the glucogenic and ketogenic amino acids, L-tyrosine, increased in SLE patients’ stool. This finding suggests that there may be impairments in metabolism of glucose energy. Metabolism of these amino acids can emerge as possible energy sources. These changes are also seen in studies of serum samples from patients (25).

Besides, the metabolite profiles of fecal specimen make it possible to distinguish SLE patients from healthy normal controls. The mixed diagnosis of phosphatidylycerol 27:2 and proline was of the biggest importance to identify SLE patients from normal and healthy controls (29).

Conclusion

Lupus heterogeneity precludes the creation of an objective tool that can be used for a wide range of patients. Identifying biomarkers for diagnosis, prognosis determining treatment response has always been one of the major challenges in this disease. In the past decades, significant developments in technology aimed to a better understanding of the risk factors and pathophysiology of SLE. Further studies can improve and increase biomarker accuracy too.

Authors’ contribution
SA and NK were principal investigators of this study. SA, NK, SMA and SMHM searched the data and prepared the primary draft. Editing the manuscript done by MM. All authors that participated in preparing the final draft of the manuscript revised the manuscript and accepted its publication.

Conflicts of interest
The authors declare that they have no competing interests.

Ethical issues
Ethical issues (including plagiarism, data fabrication and double publication) were completely observed by the authors.

Funding/Support
We have no sources of funding.

References
1. Allen ME, Rus V, Szeto GL. Leveraging heterogeneity in systemic lupus erythematosus for new therapies. Trends Mol Med. 2021;27:152-171. doi: 10.1016/j.molmed.2020.09.009.
2. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: a cellular perspective. Trends Mol Med. 2017;23:615-35. doi: 10.1016/j.molmed.2017.05.006.
3. Jarukitsopa S, Hoganson DD, Crowson CS, Sokumbi O,
Afshari S et al

Davis MD, Michet CJ Jr, et al. Epidemiology of systemic lupus erythematosus and cutaneous lupus erythematosus in a predominantly white population in the United States. Arthritis Care Res (Hoboken). 2015;67:817-28. doi: 10.1002/acr.22502.

4. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: a cellular perspective. Trends Mol Med. 2017;23:615-635. doi: 10.1016/j.molmed.2017.05.006.

5. Ali A, Sayed Z, Ameer MA, Ariif AW, Kifan F, Iftikhar A, et al. Systemic Lupus Erythematosus: An Overview Of The Disease Pathology And Its Management. Cureus. 2018;10:e3288. doi: 10.7759/cureus.3288.

6. Tuniccliffle DJ, Singh-Grewal D, Kim S, Craig JC, Tong A. Diagnosis, Monitoring, and Treatment of Systemic Lupus Erythematosus: A Systematic Review of Clinical Practice Guidelines. Arthritis Care Res (Hoboken). 2015;67:1440-52. doi: 10.1002/acr.22591.

7. La Paglia GMC, Leone MC, Leprì G, Vagelli R, Valentini E, Alunno A, et al. One year in review 2017: systemic lupus erythematosus. Clin Exp Rheumatol. 2017;35:551-561.

8. Tiao J, Feng R, Carr K, Okawa J, Werth VP. Using the American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics (SLICC) criteria to determine the diagnosis of systemic lupus erythematosus (SLE) in patients with subacute cutaneous lupus erythematosus (SCLE). J Am Acad Dermatol. 2016;74:862-9. doi: 10.1016/j.jaad.2015.12.029.

9. Kapoor RV, Vaidyanathan S. Towards quantitative mass spectrometry-based metabolomics in microbial and mammalian systems. Philos Trans A Math Phys Eng Sci. 2016;374:20150363. doi: 10.1098/rsta.2015.0363.

10. Ribbenstedt A, Ziarrusta H, Benskin JP. Development, characterization and comparisons of targeted and non-targeted metabolomics methods. PLoS One. 2018;13:e0207082. doi: 10.1371/journal.pone.0207082.

11. Vásquez-Cañizares N, Wahezi D, Putterman C. Diagnostic and prognostic tests in systemic lupus erythematosus. Best Pract Res Clin Rheumatol. 2017;31:351-363. doi: 10.1016/j.berh.2017.10.002.

12. Fiehn O. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. Curr Protoc Mol Biol. 2016;114:30.4.1-30.4.32. doi: 10.1002/0471142727.mb3004s114.

13. Marshall DD, Powers R. Beyond the paradigm: Combining mass spectrometry and nuclear magnetic resonance for metabolomics. Prog Nucl Magn Reson Spectrosc. 2017;100:1-16. doi: 10.1016/j.pmnrs.2017.01.001.

14. Li B, He X, Jia W, Li H. Novel Applications of Metabolomics in Personalized Medicine: A Mini-Review. Molecules. 2017;22:1173. doi: 10.3390/molecules22071173.

15. Krumsiek J, Bartel J, Theis FJ. Computational approaches for systems metabolomics. Curr Opin Biotechnol. 2016;39:198-206. doi: 10.1016/j.copbio.2016.04.009.

16. Gonzalez-Franquesa A, Burkart AM, Isganaitis E, Patti ME. What have metabolomics approaches taught us about type 2 diabetes? Curr Diab Rep. 2016;16:74. doi: 10.1007/s11892-016-0763-1.

17. Liu X, Locasale JW. Metabolomics: a primer. Trends Biochem Sci. 2017;42:274-284. doi: 10.1016/j.tibs.2017.01.004.

18. Markley JL, Brüschweiler R, Edison AS, EghbaliNA HR, Powers R, Raftery D, et al. The future of NMR-based metabolomics. Curr Opin Biotechnol. 2017;43:34-40. doi: 10.1016/j.copbio.2016.08.001.

19. Lei Z, Huhman DV, Sumner LW. Mass spectrometry strategies in metabolomics. J Biol Chem. 2011 Jul 22;286:25435-42. doi: 10.1074/jbc.R111.238691.

20. Boiteau RM, Hoyt DW, Nicora CD, Kinmonth-Schultz HA, Ward JK, Bingol K. Structure elucidation of unknown metabolites in metabolomics by combined NMR and MS/MS prediction. Metabolites. 2018;8:8. doi: 10.3390/metabo8010008.

21. Julià A, Alonso A, Marsal S. Metabolomics in rheumatic diseases. Int J Clin Rheumatol. 2014;9:353. doi: 10.2217/ijr.14.25.

22. Nagafuchi Y, Shoda H, Fujioka K. Immune profiling and precision medicine in systemic lupus erythematosus. Cells. 2019;8:140. doi: 10.3390/cells8020140.

23. Liang Y, Xie SB, Wu CH, Hu Y, Zhang Q, Li S, et al. Coagulation cascade and complement system in systemic lupus erythematosus. Oncotarget. 2017;9:14862-81. doi: 10.18632/oncotarget.23206.

24. Wu T, Xie C, Han J, Ye Y, Weiell J, Li Q, et al. Metabolic disturbances associated with systemic lupus erythematosus. PLoS One. 2012;7:e37210. doi: 10.1371/journal.pone.0037210.

25. Belloccchi C, Fernández-Ochoa Á, Montanelli G, Vigone B, Santaniello A, Quirantes-Piné R, et al. Identification of a shared microbiomic and metabolomic profile in systemic autoimmune diseases. J Clin Med. 2019;8:1291. doi: 10.3390/jcm80101291.

26. Alonso A, Julià A, Vinaixa M, Domènech E, Fernández-Nebro A, Cañete JD, et al. Urine metabolome profiling of immune-mediated inflammatory diseases. BMC Med. 2016;14:133. doi: 10.1186/s12916-016-0763-1.

27. Guleria A, Pratap A, Dubey D, Rawat A, Chaurasia S, Sukesh E, et al. NMR based serum metabolomics reveals a distinctive signature in patients with lupus nephritis. Sci Rep. 2016;6:35309. doi: 10.1038/srep35309.

28. Zhang Q, Yin X, Wang H, Wu X, Li X, Li Y, et al. Fecal metabolomics and potential biomarkers for systemic lupus erythematosus. Front Immunol. 2019;10:976. doi: 10.3389/fimmu.2019.00976.