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Metabolomics in the Diagnosis of Systemic Autoimmune Diseases: A Mini-Review with a Focus on Psoriatic Arthritis and Rheumatoid Arthritis

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Abstract

Complex and overlapping clinical presentation of systemic autoimmune diseases, such as Psoriatic arthritis (PsA) and Rheumatoid arthritis (RA), creates significant difficulties in early diagnosis and management. Metabolomics, as a rapidly developing field, holds a great potential to identify the intricate metabolic disturbances related to these conditions. This may assist in the early and accurate diagnosis, and personally-tailored treatment of these conditions. The current review summarizes recent metabolomics research conducted in patients with PsA and RA and employing nuclear magnetic resonance and mass spectrometry sub-techniques. The discussed studies reveal unique metabolic patterns and pathways associated with the development of PsA and RA and represent valuable information about disease pathogenesis and potential molecular-based markers. Thus, despite the substantial advances that have already been made in the field of metabolomics in systemic autoimmune diseases, there are still many gaps. First, cohort studies with larger sample sizes are required to identify and validate more metabolic abnormalities. In addition, the findings of new candidates for biomarkers must be validated, and new biofluids must be further explored for metabolomic analysis. Filling these gaps may help researchers to develop a thorough understanding of most systemic autoimmune diseases, and innovative personalized diagnostics and therapeutic strategies can be subsequently developed.

Keywords

Metabolites; biomarkers; diagnosis; biofluids; NMR; mass spectrometry

Introduction

Systemic autoimmune diseases encompass a large group of related diseases that all share a common characteristic of immune system malfunction, which causes immune cells to attack host antigens. As a result, an abundance of different tissues becomes damaged and inflamed due to inappropriate immune reactions. Systemic autoimmune diseases include some of the most burdensome to global health, with Psoriatic Arthritis (PsA) and Rheumatoid Arthritis (RA) considered the most severe in this regard(Cooper & Stroehla, 2003). Both are debilitating conditions characterized by long-term inflammation and autoimmune processes directed against joints, eventually resulting in joint destruction over time, physical disability, and quality of life impairment.

Psoriatic arthritis (PsA) is characterized by joint inflammation that occurs simultaneously with psoriasis, a common skin condition with red, scaly patches(Kumar et al., 2014; Mease et al., 2013). Some researchers define it as a complex disease and it may affect various systems such as skin and joints, ranging from mild to severe destructive polyarthritis, enthesitis, dactylitis and axial involvement(Gladman, 2015). Although exact prevalence is unknown, estimates for the population's percentage range from 0.3% to 1%(Gladman, 2005). The onset typically occurs between ages 30 and 50, and symptoms vary from mild discomfort to persistent inflammation, potentially leading to joint damage if untreated. (Gottlieb et al., 2008). So, the accurate and early diagnosis is needed for improving the quality of life. But the Diagnosing PsA is tough due to nonspecific symptoms and the absence of clear biomarkers; even during active disease, markers like erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) often appear normal(Rida & Chandran, 2020). Before 2006, there were no standardized definitions or widely accepted criteria for diagnosing PsA(Leung et al., 2018). The Moll and Wright criteria were frequently utilized to diagnose PsA as a condition associated with psoriasis and typically lacking rheumatoid factor (RF). However, these criteria did not effectively distinguish PsA, from other forms of arthritis (Helliwell & Taylor, 2005). Subsequently in 2006 the classification of psoriatic arthritis (CASPAR) group introduced and updated criteria, named as CASPAR criteria for diagnosing PsA, which included clinical serological and radiological characteristics (Taylor et al., 2006)

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune, it is distinguished by extra-articular involvement, or the involvement of areas other than the joints, and inflammatory arthritis, which causes inflammation of the joints. It is primarily affecting the synovial joints,

which are defined by the lining of synovium. Its development is frequently ascribed to the interaction of environmental and genetic factors (Chauhan et al., 2024). About five out of every 1000 adults worldwide suffer from RA, and women are about two to three times more probable than men to develop the disease(Aletaha & Smolen, 2018). The 2010 American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) classification provides guidelines for the evaluation of clinical signs and symptoms in the diagnosis of RA. Within this classification, the rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) factors are important diagnostic tools that are essential for correctly diagnosing RA.(Kay & Upchurch, 2012). Sometimes it is difficult to diagnose rheumatoid arthritis because there are no detectable levels of RF and anti-CCP levels. This condition is referred as "seronegative rheumatoid arthritis (SRA)". In such cases symptoms can over overlap with other conditions such as psoriatic arthritis, and makes diagnosis more difficult(Souto-Carneiro et al., 2020). Currently there are no specific markers for the accurate diagnosis of these two conditions. Although clinicians and experts use clinical, serological, and radiological features as the detection criteria, these techniques are not accurate due to lack of sensitivity and specificity.

Metabolomics, which has recently been developed in research, has become an essential method for early detection of the problem. This is a more critical technological breakthrough for identifying and interpreting metabolic components in cells, cell tissues, and other cell factors. It is now easier to analyze a wide range of chemicals, including carbohydrates, lipids, and amino acids. This new method can help define precision treatments and discover potential biomarkers. Data obtained from nuclear magnetic resonance and mass spectrometry of endogenous metabolites from both PsA and RA patients were compiled in this review. The summary of the metabolites for diagnosis as biomarkers is mentioned in this paper.

2. Importance of metabolomics in the disease diagnosis:

Metabolomics is a rapidly growing field in science and has attracted significant interest due to its ability to revolutionize understanding of molecular mechanisms underlying disease pathogenesis and diagnostics(Wawrzyniak et al., 2021). Primarily, metabolomics describes the systematic analysis of metabolites-small molecules produced during cellular metabolic processes within living organisms. Such metabolites incorporate a wide array of compounds including lipids, sugars, organic acids and amino acids which are essential for maintenance of normal functions within the body (Qiu et al., 2023; P. L. Yang, 2016).

Metabolomic analysis employs various approaches based on the nature of the investigative question and available analytical tools. There are two major types of methodology employed in metabolomics research; untargeted and targeted approaches(J. H. Wang et al., 2010). Untargeted approaches create a hypothesis by profiling broad range of metabolites. In this approach, all available metabolites in biological specimens are detected at once to give an overview of total metabolites which is often used to find biomarkers or identify whole metabolomes. The second method, called Targeted Metabolomics, is to quantify certain metabolites using quantitative methods that are selective for measurements of pre-designed metabolite sets from biological samples(Chen et al., 2020; López-López et al., 2018; Trezzi et al., 2015).

Metabolomics research relies on a variety of biological specimens, including serum, plasma, urine, saliva, cerebrospinal fluid (CSF), bile, seminal fluid and amniotic fluid, synovial fluid (SF) in knees and elbows and exhaled breath condensate. Tissue extracts are also used as well as blister and cyst fluids. Finally, several types of tissue biopsy samples can also be employed with accompanying lipid and aqueous extracts thereof. Specimen selection depends on the type of autoimmune disease being investigated as well as its effects on different organs. However, serum, plasma, urine and fecal extracts are mostly preferred given their non-invasive collection methods and multitude of metabolites present (Nagana Gowda et al., 2008).

Metabolomics uses primarily nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) for analysis. NMR spectroscopy, a non-destructive technique widely used in chemistry, provides detailed information on the molecular structures of single compounds and complex mixtures as well as giving quantitative data for absolute or relative concentrations. Alternatively, MS is connected to separation methodologies like liquid chromatography (LC) and gas chromatography (GC), thus helping in pre-separation. Metabolic fingerprinting is often done by MS while it is also used in identifying metabolites as well as quantifying drug metabolites(J. H. Wang et al., 2010). Notable differences between MS and NMR methodologies include sensitivity, sample preparation requirements and the sample volumes required. Specifically, MS is generally much more sensitive and can detect even trace amounts of metabolites as opposed to NMR based techniques which are limited to abundant metabolites. Furthermore, in terms of NMR-based sample preparation, it is typically simple with dilution using basic buffers often being involved. NMR-based investigations do face challenges with pH, particularly in urine samples. Finally, for a NMR analysis, larger

volumes of samples must be used compared to smaller ones used in an MS-based analysis(J. H. Wang et al., 2010)

Metabolomics is the backbone of disease diagnosis and gives a deep understanding of the metabolic changes that are associated with different diseases. In this method, researchers explore how metabolites in biological samples change over time in order to reveal processes happening within the disease state(Al-Sulaiti et al., 2023). These quantifiable compounds can answer questions about an organism's health status, complementing genomic and proteomic profiles by providing a phenotypic signature (Rahman & Schellhorn, 2023). Metabolomics enables the identification of biomarkers for different types of diseases, thus helping in early stage diagnoses of various conditions as well as prognoses and treatment improvements (Nagana Gowda et al., 2008).It also assists in separating patients' response to treatment from each other based on quantitative analysis of numerous small molecule metabolites present in biological systems for purposes of personalized drug therapy. The significance here is notable especially in such areas as cancer or diabetes where altered metabolic profiles provide vital hints on how the disease is progressing and how efficient it could be controlled (Nagana Gowda et al., 2008). With its diagnostic power being boosted by improved analytical platforms, statistical methodologies and techniques for handling data, metabolomics remains a rapidly developing science (Aderemi et al., 2021). Metabolomics offers the potential for more in-depth comprehension of metabolic intricacies in respect to health and disease, thereby improving diagnostic accuracy, through a synergistic integration of various analytical techniques such as NMR and MS.

Metabolomics is a flexible approach that can be used to appreciate the complexity in disease development and diagnosis. It allows for comprehensive profiling of metabolites, therefore permitting the simultaneous analysis of various metabolites with subtle alterations that are usually overlooked by conventional methods(Al-Sulaiti et al., 2023). This method also needs only small samples that do not have to be invasive, such as urine, tears, saliva, serum and plasma hence reducing patient suffering while at the same time cutting down health care costs. Additionally, it is reputable for its ability to identify metabolic changes necessary for early identification of diseases, risk assessment and treatment monitoring (Al-Sulaiti et al., 2023). Due to their dynamic nature, metabolic compounds make it possible to continuously observe the metabolism thus helping tracking disease progression and efficiency of treatment. When combined with other omics platforms, metabolomics offer a global view on systemic autoimmune diseases revealing intricate links between genes, environment and metabolism so as to point towards precision medicine (Al-Sulaiti et al., 2023).

3. Advancing Rheumatoid Arthritis Diagnosis through Metabolomics

Several metabolomic studies has been carried out to find rheumatoid arthritis biomarkers in different biological fluids such as serum, plasma, urine, and synovial fluid. The following table represents the disturbed metabolites and metabolic pathways discovered in the RA of an individual.

Table 1: Altered metabolites and metabolic pathways in Rheumatoid arthritis (RA)

Sample type and instrument	**Upregulated metabolites**	**Down regulated metabolites**	**Pathways effected**	**reference**
Plasma - NMR	Cholesterol C-21, acetylated glycoprotein, lactate, and unsaturated lipid	High density lipoproteins	lipid metabolism	(Lauridsen et al., 2010)
Serum – GC-MS, LC-MS	D-ribofuranose, Glyceric acid, and hypoxanthine.	Threonic acid, histidine, cholesterol methionine, threonine, and asparagine.	Amino acid metabolism, Nucleotide synthesis.	(Madsen et al., 2011)
Serum (RA VS SLE) - NMR	Glycoprotein, glucose, Lactate, VLDL and LDL.	Citrate, pyruvate, glucose, alanine, tyrosine, isoleucine, valine, phenyl alanine, lysine, histidine, glutamic acid, phosphocholine, and HDL.	Energy metabolism, amino acid metabolism, and lipid metabolism,	(Ouyang et al., 2011)
Serum (RA VS	Aspartic acid, Homoserine, Lactic	4,8-dimethylnonanoyl	TCA cycle, fatty acid metabolism,	(Jiang et al., 2013)

OA) – GC-QTOF-MS, UPLC-QTOF-MS	acid, Glyceraldehyde, and Dihydroxy fumaric acid.	carnitine	and Amino Acid metabolism	
Synovial fluid – GC-MS	lactic acid	Glucose	Energy metabolism.	(X. Y. Yang et al., 2015)
Serum - NMR	acetate, 3-hydroxy-isobutyrate, acetoacetate acetone, N-acetyl glycoprotein.	Isoleucine, valine, alanine, lactate, creatinine, and histidine, acetyl phosphocholine, sn-glycero-3-phosphocholine.	The synthesis and degradation of ketone bodies, Propanoate metabolism, glycolysis, valine, leucine and isoleucine degradation, gluconeogenesis, glycerophospholipid metabolism and pyruvate metabolism.	(Zabek et al., 2016)
Plasma – LC-MS	Lyso phosphatidyl inositol (LPI 18:2)	Alkenyl phosphatidyl ethanolamine (PE (P-36:4, 38:5, 40:5, 40:4), Alkyl phosphatidyl ethanolamine (PE-(O-36:4), phosphatidylserines (PS 36:1, PS 38:4, and PS 40:6), dihydroceramides (dhCer 22:0, 24:0),	Lipid metabolism	(Fang et al., 2016)
Urine -		Citrate, N-acetyl	TCA cycle, arginine	(Alonso et

NMR		amino acids, carnitine, and alanine	and proline metabolism	al., 2016)
Serum – GC-MS	fatty acids and cholesterol	amino acids and glucose	Fatty acid metabolism, Urea cycle, Glycolysis, Amino acid metabolism, and TCA cycle,	(Zhou et al., 2016)
Serum – LC-MS	fumaric acid, glutamic acid, glyceraldehyde, 4-methoxyphenylacetic acid, L-phenylalanine, L-tryptophan, L-leucine, L-proline, cholesterol.	Bilirubin, arginine, succinic and capric acid.	Amino acid metabolism, phospholipid metabolism and oxidative stress.	(Li et al., 2018)
Plasma - LC-TOF-MS	Ethanolamine phosphate, ATP, UTP, ADP, GDP, taurine, and 6-aminohexanoic acid.	Xanthine	Nucleotide metabolism	(Kishikawa et al., 2020)
Plasma – GC-QTOF-MS		cysteine, glutamine, citric acid	Taurine biosynthesis	(He et al., 2021)
Synovial fluid - GC-TOF-MS	Xanthine, hypoxanthine and adenosine	Citrulline, tryptophan and histidine	purine and amino acid metabolism	(Kim et al., 2022)
Serum - GC-TOF-MS	Lactic acid, 3-hydroxyisovaleric acid	Glyceric acid	Amino acid metabolism and Aminoacyl-tRNA biosynthesis.	(Rodríguez-Muguruza et al., 2023)
Serum -	Lactate, acetate,		Starch and sucrose	(Cedeno et

NMR	taurine, and o-acetyl choline		metabolism, galactose metabolism, and alanine, aspartate, and glutamate metabolism	al., 2023)

To investigate rheumatoid arthritis (RA) using serum metabolomics, we combined several approaches and important results from several studies. Initially, 3studies have found to be employed 1H-NMR technique. Each study identifies specific dysregulated metabolites or pathways associated with RA, offering potential biomarkers to enhance RA diagnosis. Notably, there is overlap in the dysregulated metabolites identified, such as valine, isoleucine, lactate, alanine, creatinine, and histidine, albeit with varying significance levels. Common findings across the studies include decreased levels of certain metabolites like valine, isoleucine, and lactate, alongside increased levels of others such as 3-hydroxyisobutyrate and acetate in RA patients compared to healthy controls(Cedeno et al., 2023; Ouyang et al., 2011; Zabek et al., 2016). Further, Zabek et al., continued the investigation on the same group of patients after 3 months of treatment and observed altered levels in dysregulated metabolites(Zabek et al., 2016). While there is overlap in the dysregulated metabolites, each study also identifies unique metabolites or metabolic pathways associated with rheumatoid arthritis. For example, the study focusing on RA and polymyalgia rheumatica (PMR) identifies lactate, o-acetylcholine, taurine, and sex (female) as distinguishable factors, which are not highlighted in the other studies (Cedeno et al., 2023). Further we explored the studies which employed the GC-MS technique to investigate the serum metabolomics. The study by Zhou employed GC-MS to analyze metabolic patterns in rheumatoid arthritis patients, finding distinct profiles with decreased amino acid and glucose levels and increased fatty acid and cholesterol levels, suggesting potential for understanding pathogenesis and for improved diagnosis. This study provided a broader characterization of the serum metabolite signature in RA (Zhou et al., 2016). While Rodríguez-Muguruza et al. extended this pursuit by comparing RA cohort (with a symptoms duration of ≤6 months and not receiving any treatment) and healthy controls and he revealed that that a panel of three metabolites successfully diagnosed 96.7% of RA patients with 94.4% specificity and 93.5% sensitivity. This study was more targeted towards developing a specific metabolite panel for early RA diagnosis(Rodríguez-Muguruza et al., 2023). Some studies employed combined mass spectrometry technique to understand the pathogenesis of RA patients using serum as a biofluid. The analysis by

Madsen et.al., was conducted comparing healthy controls, with RA patients. The preliminary results of this study were further supported by validation experiments. Compared to healthy controls, notable metabolites indicative of RA included lower levels of histidine, threonic acid, methionine, cholesterol, and asparagine and elevated levels of glyceric acid, D-ribofuranose, and hypoxanthine indicating enhanced energy metabolism (Madsen et al., 2011). Following to this, jiang et al. also employed the combined approach on 4 major types of rheumatoid arthritis. This study identified 6 unique metabolites of rheumatoid arthritis and serving as potential biomarkers for the diagnosis and stratification of RA group from OA group (Jiang et al., 2013). Further studies enriched this landscape by utilizing LC-MS to evaluate the metabolic profiles of patients with rheumatoid arthritis. totally we found two studies utilized the LC-MS approach. First study by Li et al., identified three distinct metabolites were found to be biomarkers for discriminating RA patients from healthy controls as well as from primary Sjogren's syndrome.(Li et al., 2018). Second study by Luan et al. delved into the use of machine learning-based multivariate analysis, a diagnosis model that produced results with a high level of sensitivity, specificity, and accuracy 90.2%. The model showed its efficacy for diagnosis and disease stratification by identifying both seropositive and seronegative RA patients. A set of twenty-six distinct metabolites and lipids reflecting aberrant metabolism of energy, lipids, and amino acids showed a robust correlation with disease activity and a high degree of diagnostic accuracy (Luan et al., 2021).

In addition, we expanded our research to include plasma metabolomics, to detect significant outcomes in several other studies. Scientists used 1H-NMR spectroscopy-based metabolic phenotyping to identify biomarkers for RA patients in their patients' plasma. The research included 51 healthy individuals and 47 patients with active and remission RA. The severity of the condition was determined by metabolic profiles, and the potential biomarkers identified included lipid signatures, acetylated glycoprotein, cholesterol, and lactate. The results revealed a significant difference in the metabolic profiles of individuals who suffer from active RA and those who are in remission. These results suggest that metabolic phenotyping using 1 H NMR is a novel technique for monitoring RA progression and developing a personalized RA treatment(Lauridsen et al., 2010). In the study by He and colleagues, the GC-TOF-MS technology was employed to diagnose rheumatoid arthritis through plasma metabolites. During the research, three essential metabolites were identified – L-cysteine, citric acid, and L-glutamine, as they proved to be substantially different when comparing the RA group with healthy subjects. The assessment of the subsequent validation group confirmed these findings. Moreover, an imbalance was detected within the taurine synthesis pathway concerning RA patients. According to He et al., these insights expand the knowledge

about RA's essential mechanisms and can potentially indicate new therapeutic methods(He et al., 2021). To extend, Fang et al. and Kishikawa et al. examined liquid chromatography-mass spectrometry analysed rheumatoid arthritis patients' plasma lipidomic profiles and compared them to those of healthy controls. A metabolomics study was conducted, analysing lipid classes and species. Distinct lipid profiles were distinguished among RA patients. There were still some lipids significantly correlated with the class after demographic and health confounders were taken into account, presumably reflecting a possible role in disease aetiology in this lipid metabolism dysfunction(Fang et al., 2016). A further study conducted by Kishikawa et al. was centred on the plasma nucleotides and found 8 statistically significant metabolites among RA patients relative to control. Independently of antibody status in RA patients, the above tendencies were particularly found in the patients without treatment. Thus, the plasma nucleotide levels could be considered as potential candidates for clinical biomarkers of RA, in particular in seronegative cases(Kishikawa et al., 2021).

Sasaki et al. extended the application of metabolomics as a diagnostic platform in human plasma and urine samples. The study employed quadrupole time-of-flight mass spectrometry capillary electrophoresis to discover metabolites linked to the disease activity of rheumatoid arthritis (RA). this study analysed samples from both active and inactive RA patients, identifying several metabolites that correlate with disease activity. This analysis distinguished between active and inactive RA conditions with high accuracy, guided by two key metabolites (guanidoacetic acid and histidine) in plasma and one metabolite (hypo taurine) in urine in a validation cohort. These findings point to a promising direction for RA biomarker discovery and offer deeper insights into the disease's pathophysiology(Sasaki et al., 2019).

Although synovial fluid is the direct route via which the pathological products of RA are transported, there have been few research conducted on synovial fluid because of sample collection challenges. Metabolites related to energy metabolism in the synovial fluid were analysed by yang using GC-TOF-MS technology. In contrast to the control group, RA patients had accumulating lactic acid and decreasing amount of glucose. The two patterns of proteomics were variously investigated, and the research uncovered that the HIF pathway was activated by anaerobic catabolism and aerobic oxidation dysfunction and expression disorder was mediated. This attribute can allow the RA developmental rate to be quickly accelerated, while the dysregulated metabolites could be developed as biomarkers for RA diagnosis.(X. Y. Yang et al., 2015). In a subsequent study on SF by Kim et al., the metabolites were evaluated by GC-TOF-MS method. He identified 28 significant metabolites due to the metabolism of purine and amino acids in RA and OA patients. They conducted

multiple statistical analyses which revealed that histidine, xanthine, and hypoxanthine can distinguish RA with OA perfectly. According to this study's findings, these metabolites may be employed as possible biomarkers to help with diagnosis and pathophysiology(Kim et al., 2022).

Urine metabolomics is considered quite favourable for biomarker discovery in human diseases due to its methodologies. Alonso utilized the 1H-NMR technique in urine samples of immune mediated inflammatory disease. Alonso observed a downregulation of intermediates of TCA cycle metabolites in rheumatoid arthritis. One of them, citrate, is associated with inflammation and maybe a potential target for biomarker(Alonso et al., 2016).

4. Advancing psoriatic Arthritis Diagnosis through Metabolomics

Many studies investigated the metabolic profiles of patients with RA, while the metabolomic in other subtypes of systemic autoimmune diseases, such as PsA, has been less studied. However, some attempts have been made to associate the metabolic profile of PsA patients with diagnosis and disease activity evaluation. Overall, the number of studies is smaller when comparing to those related to RA, but some candidate biomarkers for PsA have been identified, and presented in the following table.

Table 2: Altered metabolites and metabolic pathways in psoriatic arthritis (PsA)

Sample type and instrument	**Upregulated metabolites**	**Downregulated metabolite**	**Pathways effected**	**Reference**
Serum - GC-MS	Glucuronic acid, phosphoric acid, arabitol, and arabinose.			(Armstrong et al., 2014)
Serum (Ps vs PsA) – GC-MS	Arabinose, lignoceric acid, phosphoric acid, and glycerol-3-galactoside	Alpha-ketoglutaric acid	Fatty acid metabolism,	(Armstrong et al., 2014)
Urine - NMR		Citrate, alanine, carnitine, trigonelline and methyl succinate.	Amino acid metabolism,	(Alonso et al., 2016)

Plasma – UPLC-QTOF-MS and GC-FID	Lipid peroxidation products and endocannabinoids.	Phospholipids and PUFAs	Lipid metabolism	(Ambrożewicz et al., 2018)
Serum – LC-MS	TMAO			(Coras, Kavanaugh, Boyd, Huynh, Lagerborg, et al., 2019)
Serum – UPLC-MS	Pro-inflammatory eicosanoids (PGE2, 6,15-dk, dh, HXB3 and PGF1a), anti-inflammatory eicosanoids (11, 12, 15-HEPE)	anti-inflammatory eicosanoids (8,9, 11,12, 14, 15, -diHETrE, 19,20-diHDPA, 7,17 DHDPA, resolvin D1, 17-HdoHE)	Eicosanoid pathways	(Coras, Kavanaugh, Boyd, Huynh, Pedersen, et al., 2019)
PBMC – GC-MS, LC-MS/MS	8-isoPGF2α, 4-HNE, COX-2, and eicosanoids (LTB4, PGE1, TXB2, 13HODE).	15-d-PGJ2, 15-HETE	Lipid metabolism	(Wójcik et al., 2019)
Serum – LC-MS	Eicosanoids (leukotrienes, prostaglandins, and derivatives of eicosapentaenoic acid), docosahexaenoic acid, fatty acids (3-hydroxytetradecanedioic acid and 3-hydroxydodecanedioic acid)		Fatty acid metabolism.	(Looby et al., 2021)
Plasma – CE-TOF-MS, LC-	Tyramine, and saturate fatty acids	Mucic acid	Fatty acid metabolism,	(Kishikawa et al., 2021)

TOF-MS			tyramine, and vitamin related pathways	

Armstrong et al., compared the serum metabolomic profiles of psoriasis, psoriasis and psoriatic arthritis patients, and healthy controls using GC-TOF-MS Method on global metabolomics approach. They found that Patients with psoriasis and psoriatic arthritis exhibited higher glucuronic acid levels than control patients. they further idetified that lower levels of Alpha ketoglutaric acid in patients with psoriasis and psoriatic arthritis than in those with psoriasis alone. The pathophysiology of psoriasis and psoriatic arthritis is clarified by the metabolite differences, which may also offer guidance for the development of new treatments(Armstrong et al., 2014). Further, study by Coras group, explored the association between TMAO, TMA, choline metabolites and inflammation by utilizing LC-MS method. They found that only TMAO levels found to be associated with the inflammation and suggesting that TMAO plays a major role in understanding the pathogenesis of rheumatoid arthritis(Coras, Kavanaugh, Boyd, Huynh, Lagerborg, et al., 2019). Additionally, correlations between disease activity scores and levels of pro-inflammatory and anti-inflammatory eicosanoids have been reported in PsA patients by using the UPLC-MS technique. Eicosanoids are bioactive lipid mediators that regulate inflammation and immune responses. The observed changes in eicosanoid levels suggest their involvement in the inflammatory processes driving PsA pathology(Coras, Kavanaugh, Boyd, Huynh, Pedersen, et al., 2019). Furthermore, metabolomic fingerprinting using RPLC-MS was utilized in a study to identify a possible candidate marker for monitoring the disease activity and diagnosis of PsA. The results have shown that elevated levels of 1,11-undecanedicarboxylic acid compared to control acted as a diagnostic marker, and the higher levels of long-chain fatty acids were one of the predictors for severity(Looby et al., 2021). All three studies demonstrated the effectiveness of the LC-MS for the assessment of inflammatory diseases, so it can be utilized for diagnosing and understanding the causes of conditions better, which certainly will promote the technology's use in patient treatment and clinical research.

The alterations in lipid metabolism were brought to light by the serum tests. Studies on plasma also revealed that PsA patients had dysregulated lipid metabolism, which was similar with serum findings. Studies on the plasma also showed abnormalities of lipid metabolism among PsA patients, which corresponded to the serum tests. Although Ambrożewicz et al.

observed a decrease in the fatty acids and an increase in the products of their peroxidation and endocannabinoids in Ps and PsA patients. In addition, PsA patients' levels of these fatty acids were considerably lower than those of Ps patients(Ambrożewicz et al., 2018). Similarly, a recent study produced by Kishikawa and his colleagues indicated that saturated fatty acids, which are the possible primary source of the various metabolite differences between PsA and Ps(Kishikawa et al., 2021). The combination of mass spectrometry methods used in these two experiments revealed abnormalities in the metabolism of polyunsaturated fatty acids (PUFAs) and phospholipids in the plasma of individuals with psoriatic disease.

Wójcik et al. investigated mononuclear cell metabolomics in PsA and Ps patients. Monitoring the amounts of free 4-hydroxynonenal (4-HNE) and 4-HNE adducts in Ps patients' mononuclear cells may also be useful in predicting the transition from Ps to PsA, especially when paired with additional lipid monitoring. They used both LC-MS and GC-MS techniques in conjunction to detect these modifications. Patients with Ps had higher amounts of adducts, whereas those with PsA had higher levels of free 4-HNE. In addition, 4-HNE functions as a lipid mediator influencing immunological responses and inflammation and is a recognized indicator of lipid peroxidation. The observed variations in 4-HNE adduct and free 4-HNE levels point to different disease processes in PsA and Ps(Wójcik et al., 2019).

In addition to blood testing, urine analysis enables for the discovery of additional metabolic changes that may be specific to the disease in question. For example, Alonso et al. discovered that the urine citric acid concentration in PsA patients was significantly lower than that of healthy subjects, mirroring the same pattern observed in RA patients. Citric acid is a Krebs cycle metabolite required for energy metabolism and produces a large range of cellular activities. Urine citric acid is inversely related to the severity of the disease. Urine citric acid might serve as a possible RA and PsA biomarker. To determine these metabolic changes, Alonso et al. used proton nuclear magnetic resonance spectroscopy (1H-NMR), a powerful NMR-based method in metabolomics research(Alonso et al., 2016).

5. Distinguishing Metabolic Signatures Between Rheumatoid Arthritis (RA) and Psoriatic Arthritis

While RA and PsA are distinct indications, there is significant overlap in their disease presentations. They may exhibit individual differences in their metabolic profiles. The goal of this work is to differentiate between characteristics that have been identified in previous

studies on RA and PsA, making sure that these distinctions were made in identical experimental conditions.

Table 3: Comparative Studies on Metabolomic Profiles in Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA)

Sample type	Instrument	Diseased groups	Upregulated metabolites	Downregulated metabolites	reference
Serum	GC-MS and LC-MS	RA vs psoriatic arthritis	Heptanoic acid, glutamine, arabitol, inosine, succinate, pseudo uridine, guanosine, cysteine, cystine, and phosphoric acid	Glutamic acid, aspartic acid, histidine, arachidonic acid, serine, cholesterol, Monooleoylglycerol and threonic acid	(Madsen et al., 2011)
Serum	1H-NMR	PsA VS SRA	Threonine, alanine, leucine, valine, creatine, acetate, choline, and lactate.	Phenyl alanine and lipid ratios (L3/L1, L5/L1 and L6/L1)	(Souto-Carneiro et al., 2020)
fecal	LC-TOF-MS	PsA vs RA	glycerol 1-hexadecanoate, α/β-turmerone, and glutamine	Pantothenic acid, and dihydrosphingosine	(N. Wang et al., 2022)

The comparative studies by Madsen and Souto-Carneiro, delved into the serum metabolomes of RA and PsA patients, uncovering distinct concentrations of various metabolites across multiple classes. In a study by Madsen et al. employed both LC-MS and GC-MS technique and identified the dysregulation of Amino acids, organic acids, nucleosides, carbohydrates, lipids including glycerolipids, cholesterol, and fatty acids, and phosphoric acid between these two conditions(Madsen et al., 2011). Another study employed the technique 1H-NMR to investigate the plasma metabolomic and lipidomic profiles of psoriatic arthritis and seronegative rheumatoid arthritis patients. These findings revealed the variation in lipid ratios, organic compounds, and amino acids with 71% sensitivity and specificity rates by using a multivariate diagnostic model(Souto-Carneiro et al., 2020).

Furthermore, study by N. Wang et al highlighted the potential of fecal metabolites as diagnostic biomarkers for psoriatic arthritis (PsA) compared to rheumatoid arthritis (RA) and healthy controls (HCs). Using advanced analytical technique LC-QTOF-MS, this study detected 14 common metabolites have emerged as candidates for PsA diagnosis. A support vector machines model also discovered five fecal metabolites of interest as potential PsA-specific biomarkers. Pathway analysis revealed the role of amino acid metabolism, bile acid metabolism, and lipid metabolism in PsA pathogenesis (N. Wang et al., 2022).

6. Gap identification and future directions:

The objective of this review article is to summarize the recent findings from metabolomics studies specifically carried in RA and PsA and to identify areas of research that remain to be explored in the future. Most of the studies highlighted in this paper have focused more on unveiled metabolite that could be related to the diagnosis and prognosis of PsA and RA. Several metabolites were consistent in many studies when different sample types and techniques were used, but there is variability due to the heterogeneity of the samples and the methodologies were used. Efforts are being made to develop a consensus on the minimum information the evaluation of metabolomics experiment will report, which would contribute to reproducibility and the ability to compare data. It is necessary to note that the determination of metabolites is only the first step of biomarker establishment. While NMR-based platforms yield the metabolite quantities, the untargeted mass spectrometry-based methods show only relative quantitation which should be increased by targeted research for exact quantities.

Currently, the differentiation between Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) predominantly relies on biomarkers such as Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide (ACCP) antibodies. However, a subset of RA patients, known as seronegative RA (SRA), does not exhibit elevated levels of these conventional markers, complicating the diagnostic process. This complexity is worsened by a significant shared overlap of clinical, serological and radiological features between PsA and SRA (Merola et al., 2015). Nevertheless, the literature may not have sufficient studies comparing directly metabolomic profiles between PsA and SRA patients comprehensively. This condition therefore hinders the identification of disease-specific biomarkers that are necessary for an accurate diagnosis and personalized treatment. However, in spite of these efforts to discover such biomarkers they are often not tested or validated on larger more diverse populations of patients or take into account the clinical nuances and characteristics of affected individuals (Souto-Carneiro et al., 2020).

While there are no specific studies for PsA and SRA at present, the individual metabolomic profiles of PsA and SRA will need to be studied in the future as it elaborates the basic pathophysiology, and guide a more targeted therapy. These studies will focus on the unique metabolic pathways and the identification of new biomarkers for the understanding of pathogenesis and also for tailoring the specific treatment of each disease.

Also, even though metabolomic studies have made giant improvements in understanding disease mechanisms, an imbalance in the analysis of biofluids still exists. For example, blood samples have been the main focus of researchers and are certainly useful for understanding systemic metabolic changes. Nevertheless, other biofluids such as urine have not been explored widely. The advantage of using urine for metabolomics lies in its ability to capture a global non-invasive picture of whole body metabolism. The unique composition of urine containing a wide range of metabolites, ratios between them and metabolic wastes gives this fluid great potential revealing more about the pathophysiology behind systematic autoimmune diseases such as PsA and RA.

Hence, it is feasible to use metabolomics and other high tech omics technologies including genomics, transcriptomics and proteomics in order to identify the disturbed signaling pathways associated with these diseases. Moreover, it can be used along with modern methodologies for understanding systemic autoimmune diseases and developing individual biomarkers of clinical relevance.

However, the small size of current metabolomic studies limits their findings' reliability and generalizability. Although initial investigations are promising, yet lack of confirmation among a wide range of patients restricts the clinical utility of identified biomarkers. In addition, larger cohort studies should help to ascertain the robustness and applicability of metabolomic results across different population strata and clinical settings. To this end, extensive validation studies are also necessary for ascertaining whether these biomarkers have any diagnostic or prognostic value and if so make them part of our everyday medical practice.

Conclusion:

There is substantial evidence that metabolomics, an emerging cutting -edge field in science, allows a comprehensive exploration of metabolic changes related to autoimmune diseases. Through the measurement of metabolites in biological samples from patients with high-throughput technologies such as nuclear magnetic resonance and mass spectrometry, metabolomics can be used to gain insights into disease phenotypes. Metabolomics may also

help identify disease-specific biomarkers and better understand disease mechanisms. To sum up, this review provides an overview of studies of the endogenous metabolites in patients with RA and PsA across the research conducted by using NMR or MS. These studies of the metabolomic profile of PsA patients and patients with RA revealed the presence of the metabolic signature, which allows distinguishing between the metabolic patterns of each disease. Metabolites and metabolic pathways involved in the energy metabolism, lipid metabolism, amino acid metabolism, and inflammatory process were the most dis-regulated. Thus, it was shown that metabolomic analysis helps diagnose PsA and RA diseases. Further studies are needed to develop our understanding of the most common aspects of autoimmune diseases and enhance the results.

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