Review

International Journal of Clinical Rheumatology

Metabolomics in rheumatic diseases

Antonio Julià[1], Arnald Alonso[1] & Sara Marsal*[,1]

[1]Rheumatology Research Group, Vall d'Hebron Research Institute, Parc Científic de Barcelona, Torre I, 5ª Planta, c/ Baldiri i Reixac, No 4, Barcelona, 08028, Spain

*Author for correspondence: Tel.: +34 934 029 082; sara.marsal@vhir.org

Metabolites are low-weight molecules that are present in multiple biochemical processes either as intermediates or as end products of the metabolism. Consequently, the types and quantities of metabolites in a cell, tissue or organ can be informative of an underlying pathological event. Metabolomics, the global analysis of the complete metabolite profile, is a fast-developing biomedical research area. In the present article, we introduce the main methodological aspects of metabolomics and we review the most recent contributions of this approach in the study of the following rheumatic diseases: rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, psoriatic arthritis, osteoarthritis and gouty arthritis.

Keywords: bioinformatics • genomics • mass spectrometry • metabolites • metabolomics • nuclear magnetic resonance • rheumatic diseases • rheumatology

The metabolome is the most dynamic level of the organism

At the molecular level, the human body is an extremely dynamic system, with thousands of molecular reactions taking place at each instant, inside millions of cells. These biochemical reactions are responsible for maintaining the cell activity, preserving cell structure and maintaining cell-to-cell communication. For example, glucose breakdown to generate the main energy transfer molecule, ATP, is performed through a series of multiple metabolic intermediates. Each metabolite, in turn, has singular physical and chemical properties that can be used to measure its concentration at a specific time point in a certain tissue or cell type. From a biomedical perspective, the characterization of the metabolomic profile of a sample obtained from a patient can be a powerful approach to identify the physiological processes that are altered in disease. This knowledge can be key in the development of new and more effective therapeutic approaches. In addition, disease-associated metabolites can be useful biomarkers with clinically relevant applications like early diagnosis or treatment personalization.

Inflammation is a complex biological process, in which vascular, immune and other tissue-specific cell types are activated to eliminate an offending agent, either an infecting microorganism or a tissue injury. Consequently, the tissue concentrations of multiple metabolites are modified from their normal homeostatic levels. An important subset of the most severe types of rheumatic diseases is characterized by the presence of chronic inflammation, leading to tissue destruction, pain, disability and the reduction of life expectancy. Identifying the metabolomic profile associated with each clinical entity would therefore be of major importance for the development of more individualized therapeutic approaches. The recent technological and methodological advances are now allowing the fast and accurate assessment of the metabolome in many different normal and pathological conditions. In the present article we will describe these technological advances and we will review the most significant results in the metabolomics study of rheumatic diseases. Supplementary Table 1 (see online at http://www.futuremedicine.com/doi/full/10.2217/IJR.14.25) summarizes the

Future Medicine part of fsg

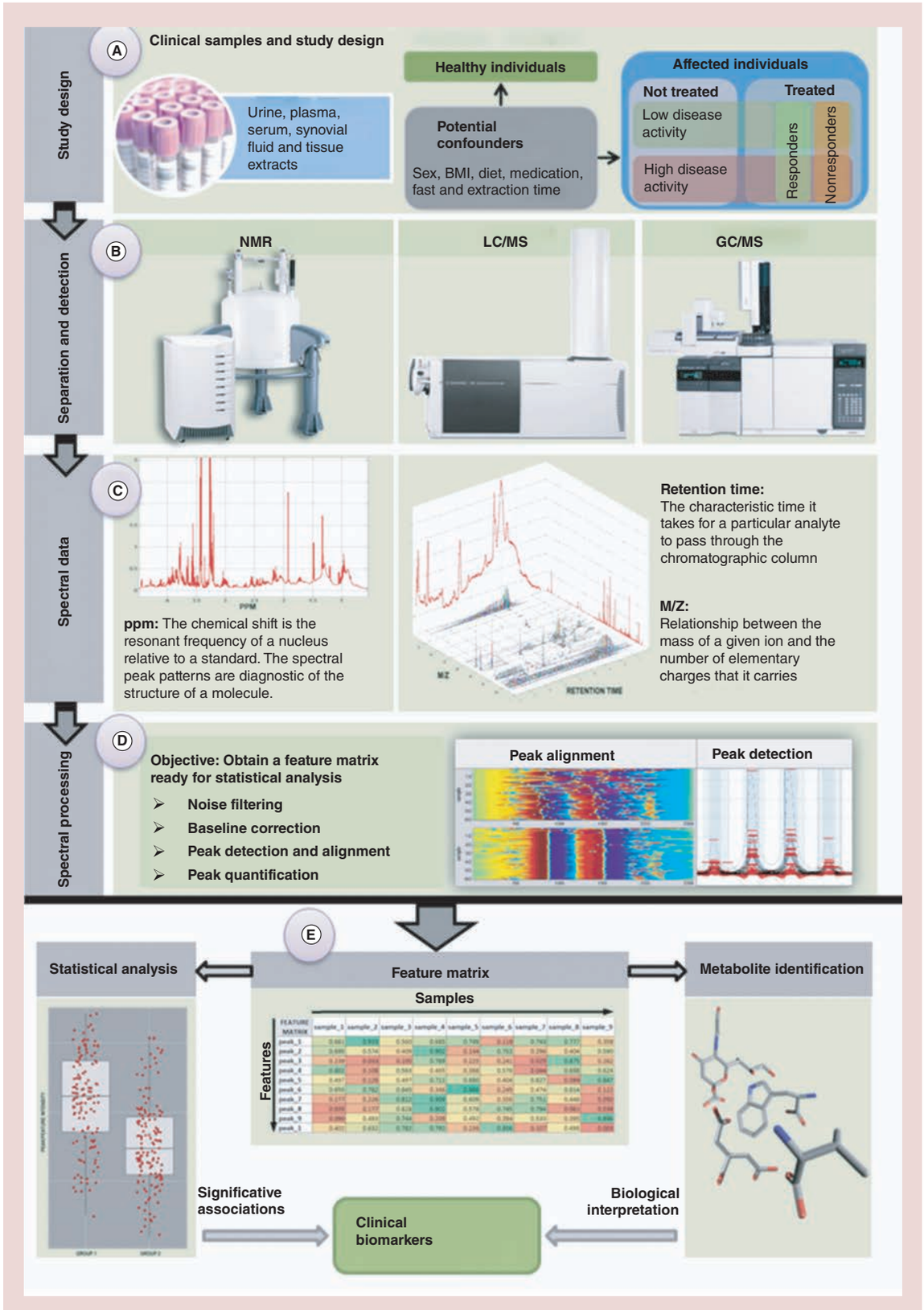
Study design
A
Clinical samples and study design
Urine, plasma, serum, synovial fluid and tissue extracts
Healthy individuals
Potential confounders
Sex, BMI, diet, medication, fast and extraction time
Affected individuals
Not treated
Treated
Low disease activity
High disease activity
Responders
Nonresponders
Separation and detection
B
NMR
LC/MS
GC/MS
Spectral data
C
PPM
ppm: The chemical shift is the resonant frequency of a nucleus relative to a standard. The spectral peak patterns are diagnostic of the structure of a molecule.
M/Z
RETENTION TIME
Retention time: The characteristic time it takes for a particular analyte to pass through the chromatographic column
M/Z: Relationship between the mass of a given ion and the number of elementary charges that it carries
Spectral processing
D
Objective: Obtain a feature matrix ready for statistical analysis
Noise filtering
Baseline correction
Peak detection and alignment
Peak quantification
Peak alignment
Peak detection
E
Statistical analysis
Feature matrix
Samples
Features
Metabolite identification
Significative associations
Clinical biomarkers
Biological interpretation

Figure 1. Metabolomics study workflow (see facing page). This figure shows the different steps associated with a metabolomics study. **(A)** According to the objectives of the study, the adequate cohorts and the type of biological samples that will be screened must be selected. **(B)** Shows the analytical instruments that are mostly used to acquire the spectral data from the biological samples. **(C)** Depending on the technological platform, the resulting spectral data are referred either in chemical shift (ppm) or in m/z and retention time. **(D)** Once the spectral data have been acquired, different processing pipelines must be applied in order to remove noise and bias and to accurately quantify each spectral peak. **(E)** This results in a feature matrix containing the quantification measures for each peak and each sample. This matrix is finally used to perform both the statistical analysis to identify significant associations and metabolite identification that will link each feature (i.e., peak) with the corresponding metabolite that will allow the biological interpretation of the identified associations.
GC/MS: Gas chromotography mass spectometry; LC/MS: Liquid chromotography mass spectometry; NMR: Nuclear magnetic resonance; ppm: Parts per million.

studies included in this review as well as the list of key metabolites identified in each disease.

Study design in metabolomic studies

There are two major study design approaches in metabolomics: targeted and untargeted studies [1,2]. In targeted studies, the researcher has a specific hypothesis to test that is based on previous knowledge of a particular biological pathway or metabolite family. In this type of studies, only a reduced set of metabolites is detected and quantified. Targeted studies are characterized for being very demanding in terms of sample preparation and analytical setup but, in exchange, metabolite measurements are sensitive and highly accurate. Consequently, these types of metabolomic studies tend to require smaller sample sizes and less bioinformatic processing steps than untargeted studies.

In untargeted studies, the goal is the measurement of the largest possible number of metabolites per sample in order to obtain a global metabolomic profile. These types of metabolomic studies are normally part of a top–down strategy, where the results obtained at the global level are used to generate new hypotheses that are subsequently validated using a targeted approach. In untargeted metabolomic studies, the large amount and complexity of the data generated require the development of highly efficient bioinformatic methods that are able to extract the most relevant biomedical information.

In metabolomics, like in many other biomedical research areas, different study designs can be considered: cross-sectional, cohort or case–control studies. Given its simplicity, one of the most commonly used study approaches in metabolomics has been the case–control design. In this design, samples from affected individuals and nonaffected individuals are drawn at random from the population at risk. With this approach, different clinically relevant comparisons can be performed to identify useful biomarkers like disease diagnostic biomarkers, disease activity biomarkers or biomarkers of drug response (Figure 1A). When using this approach in untargeted metabolomic studies of human samples, large sample sizes are generally required in order to efficiently control for potentially confounding variables like clinical and epidemiological variables (i.e., age, gender, diet or smoking status) as well as technological artifacts.

In metabolomics, the variability introduced by the observer (i.e., the biomedical researcher), can have a dramatic impact on the quality of the results. Sample manipulation, for example, is a critical step since variation at the collection, processing or sample storage steps can introduce significant biases in the resulting data. This high variability is largely due to the speed of degradation or modification of multiple metabolites in the sample. In order to minimize the negative impact of sample manipulation, the collection of human biological samples must be carefully planned and the technical variables recorded. For example, when collecting biofluids like plasma or urine the researcher must attempt to standardize influential aspects like the diet, hour of the day at which the sample is collected or the fasting time. Previous studies have shown that variation in these aspects can introduce significant biases in downstream statistical analyses [3–5]. Even seemingly irrelevant aspects like the type of containers where the samples are collected or the addition of preservatives during sample collection, can significantly alter the quality of the metabolomic data that can be obtained [4,6–8]. Consequently, the introduction of quality control measures like the standardization of all processes, the use of internal controls as well as the adequate calibration of the equipment are of paramount importance in metabolomic analysis.

Recent technological improvements are boosting metabolomic studies

In the last years, the advances in metabolomic analysis technologies as well as on bioinformatic data analysis methods have boosted the presence of metabolomics in biomedical research [2,9]. To date, the two most widely used metabolomic analysis technologies are nuclear magnetic resonance (NMR) and mass spectrometry (MS) (Figure 1B).

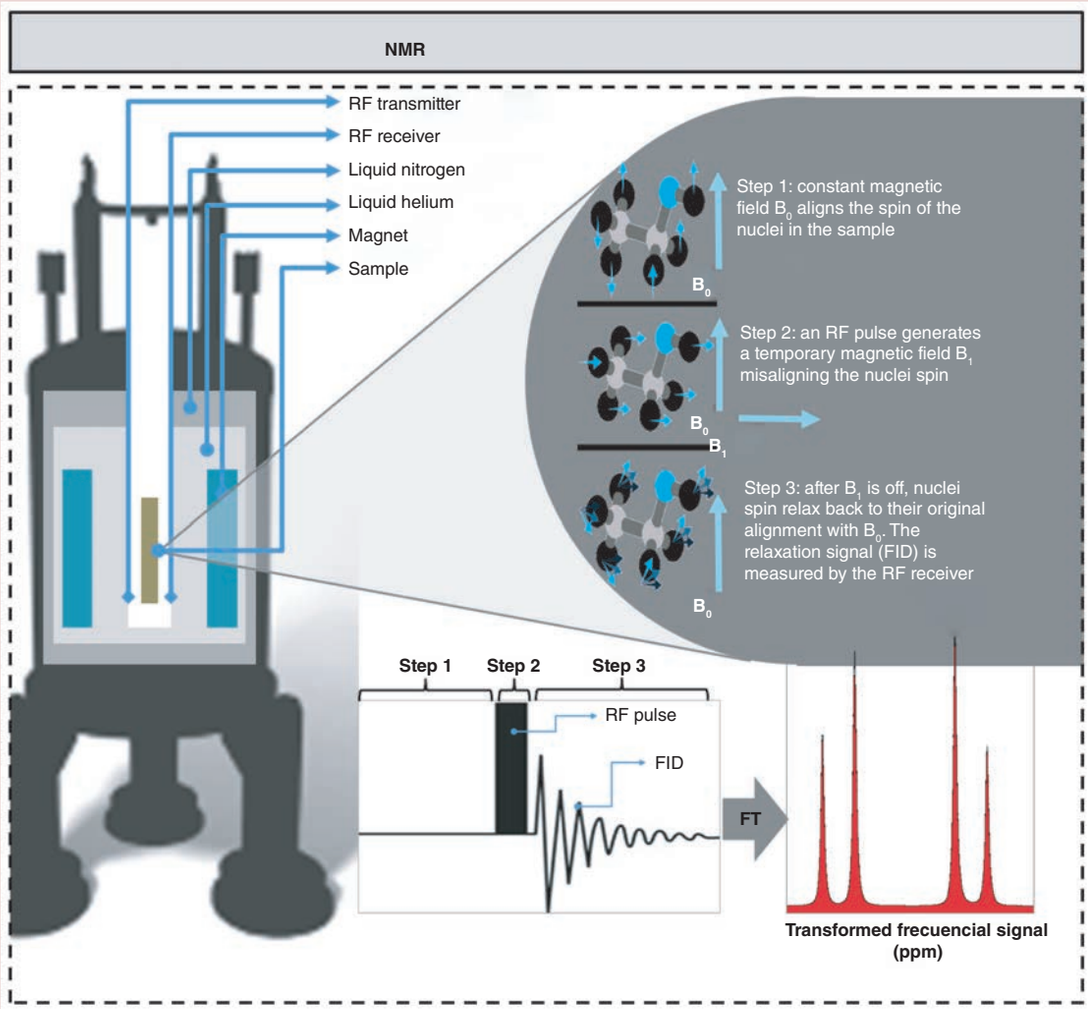

Figure 2. Analytical techniques: nuclear magnetic resonance. The NMR spectral acquisition is based on the behavior of the molecule spin under magnetic field variations. First, a constant magnetic field is applied to the sample, aligning the spins of all their molecules (i.e., step 1). The next step consists of applying a RF pulse to generate an interfering magnetic field which temporary misalign the molecules' spins (i.e., step 2). Once the interfering magnetic field disappears (i.e., step 3), the molecules' spins relax back to their original alignment. This spin relaxation results in a signal, FID, which can be measured and, after applying on the FT, is transformed on a peak spectrum where each peak is characterized by its amplitude (vertical axis) and its chemical shift (horizontal axis). The latter is usually measured in ppm and refers to the difference between the resonance frequency and that of a reference substance divided by the frequency of the spectrometer.
FID: Free induction decay; NMR: Nuclear magnetic resonance; ppm: Parts per million; RF: Radio frequency; FT: Fourier transform.

NMR is a spectroscopic analysis technique [10,11] based on the physical properties of energy absorption and re-emission of the atom nuclei due to variations in the applied magnetic field (Figure 2). Measuring the energy emitted by the atom nuclei that build up a specific molecule (i.e., free induction decay) not only allows the quantification of the concentration of the molecule itself. NMR is a fast and highly reproducible metabolomic analysis technique and has the advantage with respect to MS that it does not destroy the biological sample at study. Given its relatively low cost per sample analysis, NMR is generally the technology of choice when performing large-scale explorative studies of the metabolome.

To date, NMR-based studies have been used to identify and quantify metabolites in different types of human samples such as urine [12], serum [13] or cerebrospinal fluid [14]. Among the different NMR analytical

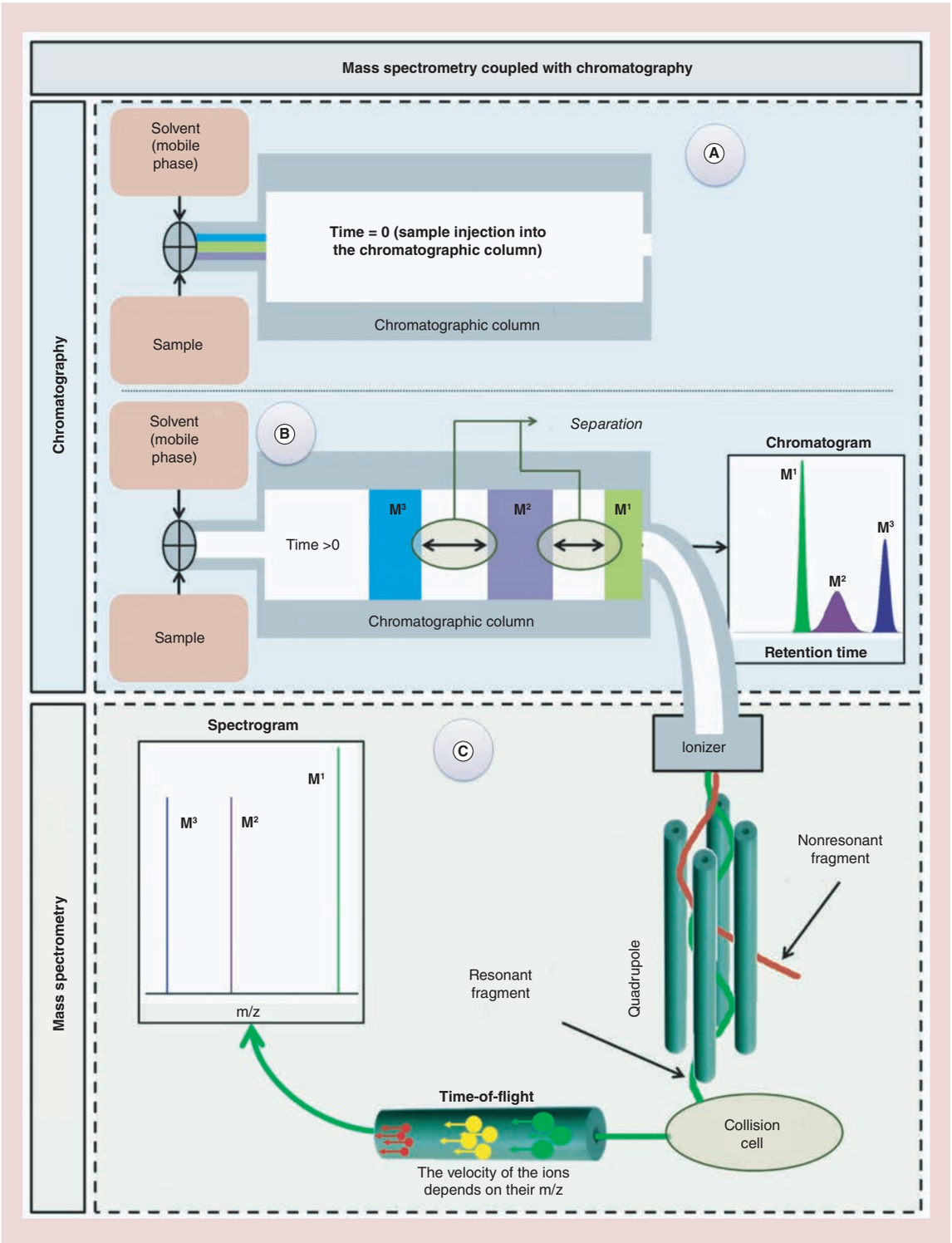

Figure 3. Analytical techniques: mass spectrometry coupled to chromatography. This figure schematizes the mass spectometry-based spectral acquisition workflow. **(A)** The sample is injected into the chromatographic column using a solvent as mobile phase. **(B)** As the sample flows through the column, the different metabolites (M^1, M^2 and M^3) are separated due to their differential retention on the stationary phase inside the column. **(C)** Once the sample has traversed the entire column, it is introduced in the mass spectrometer, which obtains the m/z of the molecules comprising the sample.
m/z: Mass-to-charge ratio.

approaches available, 1D proton NMR (^{1}H-NMR) is the most commonly used technique. The main reasons for using ^{1}H-NMR are the natural high abundance of hydrogen nuclei in metabolites, the increased sensitivity of ^{1}H-NMR compared with other NMR approaches (i.e., ^{13}C-NMR), as well as the time-efficient acquisition of spectra (see an example spectrum on Figure 1C). Together, these advantages make this technique suitable for untargeted metabolomic studies searching for biomarkers of diagnosis or prognosis, involving large sample sizes of patients and controls.

Very recently, technological advances like cryogenically cooled probes, micro-probes and increased magnetic field strength [11] are allowing a significant gain in sensitivity of NMR-based analyses. Nonetheless, NMR is unable to detect and quantify many low concentration metabolites. In order to detect and quantify this type of metabolites, MS-based technologies must be used instead.

MS is an analytical technique that generates spectral data in form of a mass-to-charge ratio (m/z) versus the relative intensity of the compounds that are generated after the ionization of the biological sample (Figure 3). The resulting ionized compounds are then measured by the MS spectrometer, generating peak signals at specific positions of the spectrum which, altogether, define the fingerprint of the original molecule. Nowadays, MS spectrometry can be performed on a broad range of instruments and techniques which use different ionization and mass selection methods [15,16]. When analyzing the metabolome, MS analysis is generally preceded by a chromatographic separation step. This step is required to reduce the high complexity of the biological sample which would otherwise be intractable. Liquid and gas chromatography columns (LC and GC, respectively) are the most commonly used chromatographic separation techniques. In both cases, metabolite separation is based on the different amount of time required by each metabolite to pass through the chromatographic column. This time, called retention time, depends on the metabolite interaction with the adsorbent material inside the column.

Compared to NMR, MS-based metabolomic analyses have a much higher sensitivity, and therefore they allow the detection and quantification metabolites that are in low concentrations in the biological samples. Compared with NMR, however, MS analyses require additional sample preparation steps [17] as well as a chromatography separation phase. For this reason, researchers performing large-scale and cost-effective exploratory studies might opt to initially use NMR techniques.

Among MS techniques, LC-MS is frequently used due to its high reproducibility and wide range of covered metabolites [18,19]. Metabolite sensitivity of LC-MS highly depends on the ionization method used (i.e., electrospray ionization is the most commonly used [20]) and also on the subsequently selected ionization mode (i.e., either positive or negative [21]). Other MS-based techniques such as GC-MS can be more reliable depending on the chemical nature of the studied metabolites (i.e., nonionizable compounds such as retinol). This technique requires the metabolites to be volatile or suited for chemical derivatization and subsequent volatilization [22,23]. Finally, the recent technological advances in LC-MS, like the use of ultra-performance liquid chromatography [20], are clearly boosting the capabilities of MS metabolomic analysis by significantly increasing specificity, sensitivity and acquisition time.

The need for bioinformatics in high-throughput metabolomic studies

The recent technological advances in metabolomic analysis have increased the quantity but also the complexity of metabolomic data that can be extracted from one single biological sample. At the same time, the development of highly specialized sample collections for 'omics' studies like biobanks are allowing the analysis of large volumes of samples. Consequently, there is a clear need for bioinformatic methods that can process this large amount of complex data and extract meaningful information in a fast and reliable way (Figure 1D).

The raw spectral data generated by NMR (Figure 1C) are known to be subject to multiple experimental and technical biases. Macro-molecule signals and other inter-sample variant factors such as used solvent, pH, ion strength and sample dilution, can produce variations on spectral peak positions and areas as well as baseline spectral artifacts [24] requiring the application of bioinformatic methods that can correct these artifacts. The two main correction methods in NMR data processing are baseline correction and peak alignment methods [25]. After these corrections are applied, two alternative bioinformatic methods can be applied to the NMR data before performing the desired statistical analysis: spectral binning and peak-based analysis. The first method is based on the automatic partitioning of the spectra in equally spaced bins. The areas calculated from each of these bins are subsequently used in the statistical analysis, in order to identify the spectral regions that are associated with the trait of interest. Limitations of this approach are that it does not account for peak positions and also that it does not exploit the presence of correlation patterns at the peak level that can be useful to identify the metabolite. In order to overcome these limitations, peak-based

methods have been developed. Making use of the correlation patterns between peaks and the information stored in the metabolite spectral databases [26], these methods can accurately identify and quantify many different metabolites present in the biological sample. In the recent years, several open-source bioinformatic methods have been developed that can partially [27,28] or completely [29] overcome all the challenges of NMR data processing.

With regards to MS-based spectral data analysis, the processing pipeline is very similar to NMR-based workflow [30]. In MS analysis, several accurate and robust bioinformatics tools have been also developed to perform MS spectra processing [31] as well as end point metabolite identification [32,33]. These tools are often dependent on the type of chromatographic column used for separation and suited for two-dimensional (i.e., retention time and m/z) spectral analysis (Figure 1C).

In addition to spectral processing and metabolite identification, high throughput metabolomic studies also require the application of complex statistical methods in order to extract relevant information (i.e., sample group clustering, biomarker identification). These multivariate analysis methods [34], also known as chemometrics methods, allow to perform tasks such as data overview, (i.e., principal component analysis), model building (i.e., partial least squares and orthogonal principal component analysis) and biomarker identification. Given the complexity associated with the metabolomics data processing and analysis, any metabolomics study will require the participation of highly trained specialists.

Metabolomic studies in rheumatoid arthritis

Since 1990, a relatively large number of studies have investigated the metabolomic changes that occur in rheumatoid arthritis (RA) pathology. Metabolomic studies in RA have evolved from using small sample sizes to investigate gross metabolic changes in changes on synovial fluid (SF) to using large sample sizes used to screen less invasive biofluids such as serum or urine, in order to disentangle the metabolome dynamics associated with disease activity and treatment response.

SF metabolome in RA

SF is a low abundance biological fluid produced from the synovial membrane filtrate of plasma and which contains high levels of hyaluronic acid. Its main purpose is the lubrication of the joint and the nutrition of the neighboring cartilage tissue. In RA, the SF is enriched with inflammatory cells, proteins and metabolites from the inflamed tissue. Therefore, the SF is a direct surrogate for the main biological processes that are taking place in the inflamed joint in RA.

The first metabolomic studies in RA were performed on SF and plasma samples from RA patients using simple NMR approaches [35–38]. The studies by Naughton *et al.* [37,38] confirmed the hypoxic nature of the inflamed synovial joint in RA, with the production of high levels of lactate compared with SF from controls. Inflammation in RA also was shown to lead to a significant consumption of lipids (i.e., reduction in low-density lipoproteins, very-low-density lipoproteins and chylomicrons), which consequently increase the concentration of ketone bodies (i.e., acetone, 3-D-hydroxybutyrate).

The anaerobic properties of RA SF were soon exploited in the search for useful disease biomarkers. Meshitsuka *et al.* [36] proposed the lactate/alanine ratio as a biomarker of RA early diagnosis since it showed increased levels compared with osteoarthritis (OA), the most common non-autoimmune arthritis. More recently, Fuchs *et al.* [39] analyzed SF and matched plasma samples of patients undergoing anti-TNF therapy. Using matrix-assisted laser desorption and ionization time-of-flight MS technology, they measured the phospholipid profile before and during anti-TNF treatment, in order to evaluate the use of plasmatic metabolites as useful markers of disease activity in the joint. They found that the ratio between phosphatidylcholine and its derivate and powerful immune chemoattractant lysophosphatidylcholine (i.e., PC/LPC ratio) that was detected in SF samples from RA patients was still detectable in their corresponding plasma samples. This aspect is of importance since it demonstrates that metabolic variations in the target tissue (i.e., SF in RA), can also be detected in plasma, which is a much less invasive type of sample. As expected, PC/LPC ratio increased with anti-TNF treatment due to the reduction of joint inflammation and the subsequent reduced production of lysophosphatidylcholine.

More recently, Giera *et al.* [40] used a more advanced technological approach to further characterize the lipid profile of RA SF. Using an untargeted LC-MS/MS system, they characterized 70 different lipid and lipid-derived metabolites. Among these metabolites they identified high levels of 5S,12S-diHETE, an isomer of LTB_4 leukotriene. 5S,12S-diHETE is produced by activated neutrophils and platelets, two cell types that have been closely related to RA pathophysiology [41,42]. The same group, using high resolution MS techniques, performed also a lipid profile analysis of RA and OA synovial samples [43]. While the unsupervised analysis of the lipid data was not able to distinguish between disease diagnostic, they were able to identify a new clustering pattern of the SF samples. A thorough analysis of this lipid profile showed that the observed

sample clustering was caused by the differential abundance of esterified oxylipids. Additional studies are needed to identify the biological origin of this family of lipids and its association with rheumatic diseases.

Serum & plasma metabolomes

In the clinical setting, blood is a convenient and useful tissue for the study of rheumatologic diseases since the collection of samples is clearly much less invasive than the sampling of the affected target tissue. In metabolomics studies of blood, the non-cellular component is separated and analyzed. When extracting the fluid component of blood, researchers can opt to use anticoagulant agents – in which case a plasma sample is obtained – or let blood clot and obtain a serum sample. The clotting of blood strongly influences the level of certain compounds like eicosanoids [13] or oxylipins. Consequently, researchers must aware of the potential impact of this non-physiologic clotting process in the results of their metabolomic studies in blood.

One of the first metabolomic approaches to the characterization of the RA serum metabolome was performed by Weljie *et al.* [44], using the K/BxN arthritic mouse model. The K/BxN model is a well-described model of inflammatory arthritis that shares many similarities with human RA. Using ^{1}H-NMR, serum samples from arthritic and control mice were compared, to identify a characteristic metabolite pattern. Uracil and TMAO were significantly increased in the serum of arthritic mice while xanthine, glycine, glycerol, hypoxanthine were significantly reduced compared to nonarthritic mice. Consequently, this study identified, for the first time, metabolites from the nucleic acid and oxidative stress pathways as potential biomarkers for RA pathology.

Using a cohort of patients and controls Lauridsen *et al.* [45] confirmed that the inflammatory state of RA is reflected in the ^{1}H-NMR spectra of human plasma samples. Similar to the discoveries in RA synovial fluid, lactate concentrations were found to be higher in patients compared with controls, probably as a consequence of the increase of the anaerobic metabolism occurring in the inflamed joints. Another likely consequence of the inflammatory activity of the synovial membrane were the high levels of acetylated glycoproteins detected in RA plasma. Finally, and in consistence with previous studies [46,47], elevated concentrations of cholesterol and low levels of high-density lipoprotein were also found to be associated with RA. High-density lipoproteins are mainly responsible for cholesterol removal from the bloodstream; together, this lipid bioprofile could explain the increased risk for coronary artery disease observed in RA patients.

Searching for a diagnostic metabolomic profile, Madsen *et al.* [48] analyzed the plasma from RA patients and compared it to controls and patients with psoriatic arthritis (PsA). In this case, a specific set of metabolites were evaluated with GC-MS and LC-MS. They observed a significant decrease of histidine levels in RA patients compared with controls and PsA patients. Low levels of this amino acid have been one of the earliest characteristic plasmatic features observed in RA [49], although its origin is still not clear. Threonic acid, a metabolite of vitamin C, was also highly expressed in RA plasma compared with controls and could be a consequence of the high oxidative stress present in the disease. Contrary to the previous study in human plasma, however, they found significantly lower levels of cholesterol in RA patients. Other studies also support the lower concentrations of cholesterol in RA compared with controls [50], so there is a clear need for additional studies to identify the potential influence of confounding variables and define the precise association of this metabolite with RA. Finally, similar to the K/BxN model screening analysis, they also found high levels of metabolites implicating the nucleotide biosynthesis (i.e., pseudouridine and guanosine) with RA. Discrepancies like the increase in hypoxanthine levels compared with the reduction in the RA mouse model remind us, however, the limitations of using animal models in the characterization of human diseases.

Very recently, Jiang *et al.* [51] used GC-MS and LC-MS to perform a metabolic analysis of the serum profile of the most prevalent forms of arthritis including RA, OA, ankylosing spondilytis (AS) and gouty arthritis (GA). Using multivariable analysis techniques on the set of measured metabolites they succeeded in discriminating all arthritis patients from healthy controls. The common arthritis profile included an increase of lactate, dihydroxyfumaric acid, glyceraldehyde, aspartic acid and homoserine, as well as a reduction in 4,8 dimethylnonanoyl carnitine. Together, the levels of these metabolites in plasma could distinguish an arthritis patient from a control individual with an 81% sensitivity and 88% specificity. In this study the differences between RA and OA (i.e., female patients) and between AS and GA (i.e., male patients) were also explored. The former comparison was based on a panel of the 13 top-ranked differential metabolites (e.g,. tryptophan, sarcosine, alanine) and yielded a classification model with an 86% sensitivity and 85% specificity. Similarly, a panel of the 16 top-ranked differential metabolites between AS and GA (e.g., creatine, cysteine, uric acid and valine) were selected to build a prediction model that reached a 79% sensitivity and 85% specificity.

The early diagnosis of RA can be crucial to the improved management of the disease and the increase

in the rates of clinical remission. For this reason, the identification of biomarkers that are informative at earlier stages of the disease can be of major importance. Using ^{1}H-NMR technology Young *et al.* [52] performed a screen in the serum metabolome of RA patients at their most initial stages and compared it to patients with more advanced disease as well as controls. Similar to Lauridsen *et al.* [45], they found low levels of lipoproteins in RA patients compared with controls. Also, the presence of 3-hydroxybutyrate, a ketone body, is in line to the previous findings of an intense anaerobic metabolism in the inflamed RA joint [37]. In the early RA group, a strong correlation of the serum metabolite profile and the degree of inflammation, represented by the levels of C-reactive protein, was found. It is therefore possible that these metabolites represent a more objective and reliable measure of the extent of the disease, including periods of apparent clinical inactivity.

Another highly relevant objective of metabolomic analysis is the identification of metabolites that are associated to disease activity and treatment response. These biomarkers could provide more objective measurements of disease activity and, therefore, allow better disease management. In their ^{1}H-NMR longitudinal analysis of human plasma, Lauridsen *et al.* [45] found that the metabolite profile of RA patients with active disease approached the profile of patients in remission after starting therapy. Importantly, both the active RA and the remission RA profiles were significantly different from the normal controls' profiles along the longitudinal study. In another longitudinal study, Madsen *et al.* [53] used GC-MS and LC-MS to identify serum metabolites correlated with the RA activity in patients starting anti-TNF therapy. In this study, the correlation of serum metabolite levels with the DAS28 disease activity score was analyzed using two independent patient cohorts. Interestingly, while highly significant linear models associated with disease activity were built in each study cohort (p-values: 6.4×10^{-6} and 9.2×10^{-3}), the predictor metabolites were quite different between both studies. A detailed analysis of the metabolite profiles of all patients suggested the existence of different underlying disease mechanisms. The existence of different RA subclasses at the molecular level has been previously suggested by whole blood transcriptomic analyses [54]. The presence of heterogeneity in RA is a clear complicating factor in the metabolomic study of the disease and consequently imposes the use of large and well-characterized patient cohorts in order to identify clinically useful biomarkers.

Urine RA metabolome

From a biomarker perspective, urine is an even more interesting biofluid than blood since it is easy to obtain and clearly non-invasive. Inflammatory diseases have shown to influence the metabolomic profile in urine [55]. Kapoor *et al.* [56] used NMR to evaluate the association of urine metabolites and the clinical outcomes of RA and PsA treated with anti-TNF therapies. For this objective, urine samples were obtained and analyzed at baseline and at 12 weeks of treatment. Multivariate analysis of the NMR spectra showed that urine metabolites could segregate RA patients with a good response from patients with a bad response to anti-TNF therapy. Among the predictor metabolites, histamine showed the highest correlation with anti-TNF response. Patients showing higher levels of this powerful cytokine were more likely to respond to anti-TNF therapy. The existence of subgroups patients showing differing levels of this metabolite could therefore explain results on its precursor histidine, which has been found to display opposing levels in different studies [48,57].

Metabolomic studies in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a variety of clinical manifestations and a wide production of auto-antibodies [58]. This marked heterogeneity makes it a challenge for clinical specialists to diagnose SLE, particularly at the first stages of the disease. Consequently, the identification of metabolomic biomarkers that can help diagnose SLE or any of its clinical subphenotypes, would be of major importance in the management of this yet incurable disease. Studies characterizing SLE metabolomic profile are very recent and have focused on the analysis of the serum and the urine metabolome.

Serum SLE metabolome

In order to identify a metabolomic profile characteristic of SLE, 2011 Ouyang *et al.* [59] performed a ^{1}H-NMR metabolomic analysis on serum samples from SLE patients and compared it to the serum profiles of controls and patients with RA. Multivariate analysis of the ^{1}H-NMR spectra showed a higher discrimination power between both rheumatic diseases and controls than between RA and SLE. This result is in accordance with previous studies demonstrating the existence a common core of metabolites in chronic inflammatory diseases. Histidine was found to have a low concentration in the serum of both RA and SLE compared with controls. Low levels of this amino acid had been also detected in the analysis of RA plasma [48]. Previous evidence in chronic kidney disease patients suggests that low histidine levels are inversely correlated with the presence of inflammation and thus could exert anti-inflammatory and antioxidant effects [60]. Other additional amino acids (alanine, tyrosine, isoleucine, valine, phenylala-

nine, lysine, histidine and glutamine) were also found to be expressed in lower levels in RA and SLE compared with controls, possibly linked to the protein turnover associated to the high inflammatory activity occurring in both diseases. Similarly, Krebs cycle metabolites citrate and pyruvate were also in low concentrations in the serum of SLE and RA patients, suggesting an association with the increase in the energy requirements in both inflammatory diseases. As found previously in RA plasma [45], SLE serum also shows reduced levels of low-density lipoproteins. The globally altered lipid profile observed in SLE patients might be an important factor in the pathogenesis of atherosclerosis in this disease [61]. Elevated levels of lactate were also found to be a powerful discriminative biomarker between in RA patients and SLE patients or controls.

In a more recent study, Xinghong *et al.* [62] used LC-MS to analyze a subset of metabolites in sera of SLE patients and controls. Multivariate analysis of the metabolite profile confirmed the clear classification of both groups. Looking for those metabolites with a stronger ability to separate SLE patients from controls, they identified an increase of proline amino acid and several lysophosphatidyl cholines as well as a decrease of phenylalanine, tryptophyan and bilirubin. Low levels of phenylalanine had been also detected in the previous study [59], suggesting a potential use as diagnostic biomarker in the early phases of SLE.

Using also MS technologies, Wu *et al.* [63] performed a case–control study to find additional serum metabolites associated with SLE. Importantly, in this study they included an additional group of cases and controls to validate the metabolites associated with SLE in the initial (discovery) cohort. In this study they identified a marked increase in lipid peroxidation products like 9-HODE and 13-HODE. Importantly, the level of these metabolites correlated with the increase of disease activity in patients, indicating a potential applicability as biomarkers. In concordance with this rise in oxidative stress, the levels of glutathione were also reduced in SLE patients. Vitamin B6, which is necessary for the production of glutathione, was found to be significantly reduced in SLE patients' sera, which might explain the reduction of this powerful antioxidant. This finding indicates vitamin supplementation could be a potential adjunctive therapy to reduce oxidative damage in SLE. While most differential metabolites were found to be reduced in SLE patients, two metabolites associated with the leukotriene production pathway, leukotriene B4 and 5-HETE, were found to be significantly overexpressed in serum.

Urine SLE metabolome

Renal involvement is the strongest predictor of morbidity and mortality in SLE and, consequently, there is major need to identify useful biomarkers associated to this severe outcome. However, while lupus nephritis has been largely studied from a proteomics perspective [64–67], metabolomic studies on lupus nephritis are still infrequent. Recently, Romick *et al.* [68] used ^{1}H-NMR to perform a metabolomic screen of urine to identify metabolites that can help discriminate proliferative, pure membranous and focal segmental glomerulosclerosis in SLE. They found that taurine and citrate, which have been previously associated to tubular renal function [69], were strong biomarkers distinguishing proliferative (classes III/IV) from pure membranous classes (class V). While class III/IV patients had low taurine levels and normal citrate levels in urine, class V patients had low citrate levels and high taurine levels. These differences lead to an almost perfect discrimination between the two lupus nephritis subtypes. The plasmatic levels of the two metabolites are known to be regulated by the kidney. Pathological differences between both SLE nephritis subclasses might explain the differential amount of metabolite finally excreted to urine.

Brain SLE metabolome

Compared to other rheumatic diseases, the nervous system is frequently affected in SLE patients leading to neuropsychiatric syndromes [70]. An altered glucose metabolism in the brain has been associated to the development of these psychiatric symptoms in SLE patients [71]. Using a well known SLE mouse model, Alexander *et al.* [71] used ^{13}C NMR and ^{1}H-NMR to evaluate the incorporation of glucose in brain extracts compared with control mice. The results clearly confirmed the altered brain metabolism in SLE. Choline was found to be highly increased in the brains of the diseased mice. Choline is a precursor for the synthesis of phospholipids and it is known to participate in inflammation by contributing to the production of arachidonic acid [72] which, in turn, leads to the increase of prostaglandins which exert multiple roles in the inflammatory response [73]. Glutamate and glutamine were also significantly increased and, together, suggest a predominant role for the glial cells (astrocytes and microglia) rather than neurons in the pathological events in the SLE brain. Also, lactate levels were found to be increased; lactate might be a product of infiltrating macrophages in the inflamed brain and it could also contribute to the alteration of the brain functionality in SLE.

Metabolomic studies in AS

AS is a chronic inflammatory disease characterized by axial skeleton ankylosis, enthesitis inflammation and, occasionally, peripheral arthritis. AS has an overall incidence between 0.5 and 14 per 100,000 people per

year and is more common in men [74]. Metabolomic studies in AS are very recent and have been all performed in blood samples with the final objective of detecting diagnostic biomarkers [51,75,76].

AS blood metabolome

Gao *et al.* [76] performed a case–control study using GC-MS an LC-MS to identify AS biomarkers in plasma samples. Supervised partial least squares discriminant analysis was able to accurately discriminate both samples groups demonstrating the potential of metabolomics as a reliable diagnostic tool in AS. In this study, proline, glucose, phosphate, phenylalanine, urea, glycerol and homocysteine were detected at higher concentrations in AS patients than in healthy controls. Instead, propanoic acid, tryptophan and several phosphatidylcholines were present at lower concentrations in AS patients compared with controls. Tryptophan reduction might respond to the indoleamine 2,3-dioxygenase enzyme activation by the high levels of interferon gamma produced by the disease. AS patients responding to anti-inflammatory treatments have shown to increase this amino acid and, consequently, it could become a useful biomarker of disease activity. Cartilage breakdown by the chronic inflammation in AS could explain the observed high levels of proline. Consequently, the high levels of urea would therefore caused by the rise in this and additional amino acids detected in AS patients.

Using LC-MS, Fischer *et al.* [75] also performed a case–control study with serum samples of AS patients and healthy controls. Like Gao *et al.* [76], multivariate analysis on the measured metabolomic profile was able to distinguish patients from controls. Importantly, the metabolomic data could also separate AS patients according to the Bath Ankylosing Spondylitis Disease Activity Index (i.e., BASDAI). Although most of the associated molecular features detected by LC-MS were not linked to known metabolites, they were able to identify 25-hydroxyvitamin D3 26,23-peroxylactone as a metabolite clearly downregulated in AS. The lower levels of this metabolite might reflect an alteration in the vitamin D3 metabolism, which has been shown to have profound effects in bone remodeling and immune cell activation [77]. Consequently, targeting this biological pathway could have protective effects in the bone destruction process associated to AS.

Finally, the arthritis screening study performed by Jiang *et al.* [51] provided additional support to the utility of serum as a useful surrogate of AS pathology. Using GC-MS and LC-MS on male subjects, they were able to identify several metabolites that could efficiently distinguish AS from GA patients. Creatinine, uric acid, arabitol, succinic acid, valine and 5-oxoproline were among the metabolites found to be significantly underexpressed in AS serum compared with GA serum.

Metabolomic studies in PsA

PsA is an inflammatory arthritis that is associated with psoriasis. PsA has specific clinical features such as arthritis of the distal interphalangeal joints to spondylitis and sacroiliitis. PsA occurs in approximately 12% of psoriasis patients [78] and it is associated with higher morbidity and mortality and also requires a markedly different therapeutic approach. Therefore, the identification of metabolites that can characterize PsA from purely cutaneous psoriasis could become a useful clinical tool. To date, however, no studies have directly compared PsA and psoriasis metabolomics profiles.

PsA blood metabolome

In their MS screen of RA, Madsen *et al.* [48] included a PsA cohort as an additional control group to evaluate the specificity of the metabolomic markers. Interestingly, the metabolomic profiles from RA patients were found to be more different between RA and PsA patients than RA and control patients. In this study, however, no direct contrast between PsA and controls was performed. Consequently, the identification of metabolites specifically associated with PsA can only be extrapolated from the differences between RA and controls and RA and PsA patients. Several amino acids including aspartic acid, glutamic acid, glutamate and serine were increased in PsA patients compared with RA and were not different between RA and controls. Therefore, it is likely that PsA is characterized by a major protein turnover and a higher increase of free amino acids in serum. However, specific case–control design studies must still be carried out to confirm the specificity of this metabolomic profile in PsA.

PsA urine metabolome

In their NMR longitudinal study of the urine metabolite profile associated to the response to anti-TNF treatment, Kapoor *et al.* [56] also included a cohort of PsA patients. Like for RA patients, the urine metabolome in PsA correlated with the changes in disease activity induced by the biologic treatment. The metabolite levels influenced by anti-TNF treatment were found to be similar between both diseases. In particular, high levels of glutamine, phenylacetic acid and histamine in the baseline urine samples were found to be predictors of the good response to anti-TNF treatment.

Metabolomic studies in OA

OA is the most common type of arthritis and is a major cause of pain and disability in the elderly [79]. The clin-

ical and radiologic findings that form the basis of the diagnosis of OA are poorly sensitive for monitoring the progression of the disease. Consequently, the identification of metabolites that can better reflect quantitative and dynamic changes of the joint tissue turnover would be of major utility in daily clinical practice.

OA urine metabolome

A first approach to the characterization of metabolites associated to OA pathology was performed by Lamers *et al.* analyzing the urine profiles of animal models with ^{1}H-NMR [80]. The Hartley outbred strain of guinea pigs has shown to develop progressive knee OA and, consequently, are a useful animal model to screen for potential biomarkers in OA. Principal component analysis, a multivariate approach commonly used in genomic studies, showed a clear separation between the urine profile of the OA model and the urine profile of the control strain. The major changes associated with OA were found in lactate, malate, hypoxanthine and alanine levels, which support the hypothesis that in OA there is an increase in energy utilization and an altered metabolism of purines. Having proven the existence of a urine metabolite pattern correlated with the presence of the disease, the same group performed a study using a cohort of OA patients and matched controls. Using the same multivariate analysis approach as in the animal model they were also able to discriminate between patients and controls. The prediction model built from the metabolite concentrations showed a strong correlation with the Kellgren–Lawrence radiographic scores for the evaluation of OA severity. Additionally, the metabolite profile that characterized the human OA patients shared many features with the profile obtained in the guinea pig model, confirming the usefulness of this model to pursue clinically relevant biomarkers. Like in the animal model, several of the differential NMR signals could not be identified. This aspect is characteristic of the exploratory nature of ^{1}H-NMR; in these cases, additional studies using more sensitive technologies like correlation spectroscopy NMR, LC-MS or GS-MS are required to characterize the associated metabolites. The increased metabolites in OA that could be identified with certainty were hydroxybutyrate, pyruvate, creatine/creatinine and glycerol. These results suggest the increased use of fat as an energy source in OA. Histidine and methylhistidine were found to be in significant lower concentration in the urine OA patients compared with controls. Low levels of histidine in OA could be associated to an over expression of histidine decarboxylase in OA chondrocytes and the subsequent increase in the production of histamine observed in the OA joint [81]. There is evidence supporting that histamine promotes the formation of the chondrocyte clusters characteristic of the osteoarthritic cartilage [82].

OA blood metabolome

Using ^{1}H-NMR Zhai *et al.* [83] analyzed the metabolomics profile in the serum of OA patients and controls. Importantly, in this study an independent replication cohort of patients and controls was also recruited to validate the metabolite associations identified in the discovery phase. Also, in this study they also used the ratios between metabolites since it has been shown to provide an improved quantification of some of the metabolic reactions present in the tissue of interest. After correcting for the number of statistical tests performed, they found the valine/histidine and the xleucine(isoleucine and leucine)/histidine ratios to be significantly associated with the presence of knee OA. These ratios showed also to be predictive of the OA severity, showing a correlation with the Kellgren–Lawrence OA grade. Interestingly, valine, isoleucine and leucine belong to the branched-chain family of amino acids (BCAA). BCAA are characterized for being essential amino acids (i.e., they cannot be synthesized by the body), having a similar molecular structure and being important constituents of the skeletal muscle. Elevated levels of BCAA have been also found both associated to obesity [84] and aging [85]. Importantly, BMI and age were discarded as confounders from the observed association between the two ratios and knee OA. Another potential explanation for the increased levels of these amino acids could be the collagen breakdown that is associated with this disease. A recent study comparing the metabolomic profile of media conditioned by cultured synovial fibroblasts from OA patients and controls also found additional evidence implicating the BCAA metabolism with OA etiology [86]. Consequently, the BCAA/histidine ratio could become a valuable biomarker in the management of OA.

Metabolomic studies in GA

Approximately 1% of the adult men in western countries have gout. GA is characterized by recurrent attacks of acute monoarticular or oligoarticular inflammation caused by the formation of urate crystals in the joint [87]. Misdiagnosis in the early stages of the disease can influence the outcome of the disease. Consequently, there is a need to identify biomarkers that can help to reliably diagnose the disease.

GA metabolome

Liu *et al.* [88] used high performance liquid chromatography to analyze both the serum and urine profiles of GA patients and matched controls. The multivariate

analysis of the obtained profiles was able to discriminate between the case and controls groups. Patients with GA showed increased serum levels of uric acid, creatinine and tryptophan. Uric acid is the end product of purine degradation and, in high concentrations, it leads to the formation of monosodium urate crystals in the joint. Although this is a well established pathological process of GA, this study shows that while it uric acid could be an informative biomarker at the earlier stages of the disease, it is not sufficiently informative

Executive summary

The metabolome is the most dynamic level of the organism

- The characterization of the metabolomic profile of patients can be a powerful approach to identify the physiological processes that are altered in disease.
- The recent technological and methodological advances are now allowing the fast and accurate assessment of the metabolome.

Study design in metabolomic studies

- There are two major study design approaches in metabolomics: targeted and untargeted studies.
- Given its simplicity, one of the most commonly used study approaches in metabolomics has been the case–control design.
- In metabolomics, the technical and biological variabilites can have a dramatic impact on the quality of the results and must be controlled.

Recent technological improvements are boosting metabolomic studies

- The two most widely used metabolomic analysis technologies are nuclear magnetic resonance (NMR) and mass spectrometry (MS).
- NMR is a fast and highly reproducible metabolomic analysis.
- MS has a higher sensitivity than NMR, but is more demanding in terms of sample preparation and technical requirements.

The need for bioinformatics in high-throughput metabolomic studies

- There is a clear need for bioinformatic methods that can process these large amounts of complex data in metabolomic studies and extract meaningful information.
- In the recent years, several open-source bioinformatic methods have been developed that can overcome several challenges of NMR and MS data processing.

Metabolomic studies in rheumatoid arthritis

- Metabolomic studies confirm the hypoxic nature of the inflamed synovial joint in rheumatoid arthritis (RA).
- Metabolite profiles in RA plasma are associated to disease activity and treatment response.
- Metabolites from the nucleic acid and oxidative stress pathways are potential biomarkers for RA pathology.

Metabolomic studies in systemic lupus erythematosus

- Low histidine levels have been identified in the serum of systemic lupus erythematosus (SLE) and RA.
- The commonly altered lipid profile in SLE and RA could explain the increased incidence of cardiovascular disease observed for both rheumatic diseases.
- Taurine and citrate levels in the SLE urine metabolome have potential utility as biomarkers for SLE nephritis subtype discrimination.

Metabolomic studies in ankylosing spondylitis

- Metabolomic studies of patients and controls identified reduced levels of tryptophan in ankylosing spondylitis, probably due to IFN-γ expression in the disease.
- Metabolite levels in serum reflect an alteration in the vitamin D3 metabolism in ankylosing spondylitis.

Metabolomic studies in psoriatic arthritis

- To date, no studies have directly compared psoriatic arthritis and psoriasis metabolomics profiles.
- The urine metabolome in psoriatic arthritis is correlated with the changes in disease activity induced by anti-TNF treatment.

Metabolomic studies in osteoarthritis

- The prediction model built from the urine metabolite concentrations correlates significantly with the Kellgren–Lawrence radiographic scores of osteoarthritis severity.
- The valine/histidine and the xleucine(isoleucine and leucine)/histidine ratios are potential biomarkers of the development of knee osteoarthritis.

Metabolomic studies in gouty arthritis

- Uric acid is not a sufficiently informative biomarker of gouty arthritis and additional markers must be identified.
- Gouty arthritis also expresses the common core of serum metabolites found in other prevalent arthritis. This common set of metabolites could be useful for the development of improved diagnostic systems.

to be used as a single diagnostic marker. Creatinine, a widely used biomarker of the renal function, could be associated to the renal affection by the deposition of urate crystals. Compared to RA, AS or SLE, where tryptophan serum levels are significantly reduced, it appears to be significantly increased in GA. The origin of this variation is not clear and, while it could be associated to GA pathology, it could also be a metabolism product resulting from the treatment received by the patients. Therapies can therefore be a major confounder in metabolomic studies if inadequately controlled.

Interestingly, uric acid and creatinine levels in urine showed an opposite variation compared with serum of GA patients. This inverse relation could be explained by the defects of tubular secretion associated with this disease. Hippuric acid, a conjugate of benzoic acid and glycine normally generated by microfloral metabolism, was also found to be reduced in GA urine. The authors, however, suggest that the increased energy consumption associated with GA associated inflammation could be responsible for this observed metabolite reduction.

In their recent screen of the serum metabolomic profiles of four different types of arthritis, Jiang [51], confirmed the high diagnostic utility of uric acid and creatine for GA. Also, additional metabolites like cystine, arabitol and alloxanoic acid were found to be in high concentrations in patients with GA. Used together in a multivariate model, they could clearly distinguish GA patients from AS patients or controls. The results of this study are a strong basis for the development of diagnostic systems based on the screening of multiple informative biomarkers.

Future perspective

Recent technological advances have boosted the capacity to mine the metabolite composition of biological samples associated with different diseases. This new layer of information strongly complements the previously established genomic, transcriptomic and proteomic technologies. In the near future, studies integrating these different layers of biological information will provide essential knowledge for the identification of the biological mechanisms that operate in each disease and will provide an accurate molecular profile of each patient. This individual profile will have a high translational potential in rheumatic disease since it could help to advance the time of diagnosis as well as help medical specialists to perform more guided therapeutic decisions.

There are still, however, several challenges that need to be overcome. The annotation of many metabolites must clearly evolve, a task that is actually being carried out by different databases [26,89,90]. Metabolome analysis technologies must improve in sensitivity and be less time consuming and costly and the associated analysis algorithms must improve their accuracy. Also, if large cohort analyses are to be performed, there is a clear need for improvement in the throughput of most metabolomics platforms. Additionally, sample and clinical collection procedures must be standardized to ensure the quality of the results and the minimization of technical and biological confounders. Although metabolomics is an emergent discipline, it is rapidly evolving and, in the next years, new findings will clearly increase our knowledge of the molecular basis of rheumatic diseases and contribute to improve the prognosis of these patients.

Financial & competing interests disclosure

This work was supported by the Spanish Ministry of Economy and Competitiveness, grants PI12/01362, PSE-010000-2006-6 and IPT-010000-2010-36, and by the AGAUR FI grant (2013/00974). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1 Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem. Soc. Rev.* 40(1), 387–426 (2011).

2 Patti GJ, Yanes O, Siuzdak G. Innovation: metabolomics: the apogee of the omics trilogy. *Nat. Rev. Mol. Cell Biol.* 13(4), 263–269 (2012).

3 Rasmussen L, Savorani F, Larsen T, Dragsted L, Astrup A, Engelsen S. Standardization of factors that influence human urine metabolomics. *Metabolomics* 7(1), 71–83 (2011).

4 Townsend MK, Clish CB, Kraft P *et al.* Reproducibility of metabolomic profiles among men and women in 2 large cohort studies. *Clin. Chem.* 59(11), 1657–1667 (2013).

5 Winnike JH, Busby MG, Watkins PB, O'Connell TM. Effects of a prolonged standardized diet on normalizing the human metabolome. *Am. J. Clin. Nutr.* 90(6), 1496–1501 (2009).

6 Bando K, Kawahara R, Kunimatsu T *et al.* Influences of biofluid sample collection and handling procedures on GC-MS based metabolomic studies. *J. Biosci. Bioeng.* 110(4), 491–499 (2010).

7 Lauridsen M, Hansen SH, Jaroszewski JW, Cornett C. Human urine as test material in 1H NMR-based metabonomics: recommendations for sample preparation and storage. *Anal. Chem.* 79(3), 1181–1186 (2007).

8 Want EJ, Wilson ID, Gika H *et al.* Global metabolic profiling procedures for urine using UPLC-MS. *Nat. Protoc.* 5(6), 1005–1018 (2010).

9 Zhang A, Sun H, Wang P, Han Y, Wang X. Modern analytical techniques in metabolomics analysis. *Analyst* 137(2), 293–300 (2012).

10 Bothwell JH, Griffin JL. An introduction to biological nuclear magnetic resonance spectroscopy. *Biol. Rev. Camb. Philos. Soc.* 86(2), 493–510 (2011).

11 Emwas A-H, Salek R, Griffin J, Merzaban J. NMR-based metabolomics in human disease diagnosis: applications, limitations, and recommendations. *Metabolomics* 9(5), 1048–1072 (2013).

12 Bouatra S, Aziat F, Mandal R *et al.* The human urine metabolome. *PLoS ONE* 8(9), e73076 (2013).

13 Psychogios N, Hau DD, Peng J *et al.* The human serum metabolome. *PLoS ONE* 6(2), e16957 (2011).

14 Wishart DS, Lewis MJ, Morrissey JA *et al.* The human cerebrospinal fluid metabolome. *J. Chromatogr. B* 871(2), 164–173 (2008).

15 El-Aneed A, Cohen A, Banoub J. Mass spectrometry, review of the Basics: electrospray, MALDI, and commonly used mass analyzers. *Appl. Spectrosc. Rev.* 44(3), 210–230 (2009).

16 Marshall AG, Hendrickson CL. High-resolution mass spectrometers. *Annu. Rev. Anal. Chem.* 1(1), 579–599 (2008).

17 Vuckovic D. Current trends and challenges in sample preparation for global metabolomics using liquid chromatography–mass spectrometry. *Anal. Bioanal. Chem.* 403(6), 1523–1548 (2012).

18 Gika HG, Theodoridis GA, Wilson ID. Liquid chromatography and ultra-performance liquid chromatography–mass spectrometry fingerprinting of human urine: sample stability under different handling and storage conditions for metabonomics studies. *J. Chromatogr. A* 1189(1–2), 314–322 (2008).

19 Theodoridis GA, Gika HG, Want EJ, Wilson ID. Liquid chromatography–mass spectrometry based global metabolite profiling: a review. *Anal. Chim. Acta* 711(0), 7–16 (2012).

20 Denoroy L, Zimmer L, Renaud B, Parrot S. Ultra high performance liquid chromatography as a tool for the discovery and the analysis of biomarkers of diseases: a review. *J. Chromatogr. B* 927(0), 37–53 (2013).

21 Nordström A, Want E, Northen T, Lehtiö J, Siuzdak G. Multiple ionization mass spectrometry strategy used to reveal the complexity of metabolomics. *Anal. Chem.* 80(2), 421–429 (2007).

22 Koek M, Jellema R, Greef J, Tas A, Hankemeier T. Quantitative metabolomics based on gas chromatography mass spectrometry: status and perspectives. *Metabolomics* 7(3), 307–328 (2011).

23 Tsugawa H, Bamba T, Shinohara M, Nishiumi S, Yoshida M, Fukusaki E. Practical non-targeted gas chromatography/mass spectrometry-based metabolomics platform for metabolic phenotype analysis. *J. Biosci Bioeng.* 112(3), 292–298 (2011).

24 Xiao C, Hao F, Qin X, Wang Y, Tang H. An optimized buffer system for NMR-based urinary metabonomics with effective pH control, chemical shift consistency and dilution minimization. *Analyst* 134(5), 916–925 (2009).

25 Zhang S, Nagana Gowda GA, Ye T, Raftery D. Advances in NMR-based biofluid analysis and metabolite profiling. *Analyst* 135(7), 1490–1498 (2010).

26 Wishart DS, Jewison T, Guo AC *et al.* HMDB 3.0 – the Human Metabolome Database in 2013. *Nucleic Acids Res.* 41(D1), D801–D807 (2013).

27 Hao J, Astle W, De Iorio M, Ebbels TMD. BATMAN – an R package for the automated quantification of metabolites from nuclear magnetic resonance spectra using a Bayesian model. *Bioinformatics* 28(15), 2088–2090 (2012).

28 Ludwig C, Gunther U. MetaboLab – advanced NMR data processing and analysis for metabolomics. *BMC Bioinform.* 12(1), 366 (2011).

29 Alonso A, Rodríguez MA, Vinaixa M *et al.* Focus: a robust workflow for one-dimensional NMR spectral analysis. *Anal. Chem.* 86(2), 1160–1169 (2013).

30 Castillo S, Gopalacharyulu P, Yetukuri L, Orešič M. Algorithms and tools for the preprocessing of LC–MS metabolomics data. *Chemometr. Intel. Lab. Syst.* 108(1), 23–32 (2011).

31 Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal. Chem.* 78(3), 779–787 (2006).

32 Alonso A, Julià A, Beltran A *et al.* AStream: an R package for annotating LC/MS metabolomic data. *Bioinformatics* (2011).

33 Kuhl C, Tautenhahn R, Böttcher C, Larson TR, Neumann S. CAMERA: an integrated strategy for compound spectra extraction and annotation of liquid chromatography/mass spectrometry data sets. *Anal. Chem.* 84(1), 283–289 (2011).

34 Madsen R, Lundstedt T, Trygg J. Chemometrics in metabolomics – a review in human disease diagnosis. *Analyt. Chim. Acta* 659(1–2), 23–33 (2010).

35 Claxson A, Grootveld M, Chander C *et al.* Examination of the metabolic status of rat air pouch inflammatory exudate by high field proton NMR spectroscopy. *Biochim. Biophys. Acta (BBA)* 1454(1), 57–70 (1999).

36 Meshitsuka S, Yamazaki E, Inoue M, Hagino H, Teshima R, Yamamoto K. Nuclear magnetic resonance studies of synovial fluids from patients with rheumatoid arthritis and osteoarthritis. *Clin. Chim. Acta* 281(1–2), 163–167 (1999).

37 Naughton D, Whelan M, Smith EC, Williams R, Blake DR, Grootveld M. An investigation of the abnormal metabolic status of synovial fluid from patients with rheumatoid arthritis by high field proton nuclear magnetic resonance spectroscopy. *FEBS Lett.* 317(1–2), 135–138 (1993).

38 Naughton DP, Haywood R, Blake DR, Edmonds S, Hawkes GE, Grootveld M. A comparative evaluation of the metabolic profiles of normal and inflammatory knee-joint synovial fluids by high resolution proton NMR spectroscopy. *FEBS Lett.* 332(3), 221–225 (1993).

39 Fuchs B, Schiller J, Wagner U, Häntzschel H, Arnold K. The phosphatidylcholine/lysophosphatidylcholine ratio in human plasma is an indicator of the severity of rheumatoid arthritis:

investigations by 31P NMR and MALDI-TOF MS. *Clin. Biochem.* 38(10), 925–933 (2005).

- **Proves the utility of plasma as a useful surrogate tissue to capture the activity of rheumatoid arthritis (RA).**

40 Giera M, Ioan-Facsinay A, Toes R *et al.* Lipid and lipid mediator profiling of human synovial fluid in rheumatoid arthritis patients by means of LC-MS/MS. *Biochim Biophys Acta.* 1821(11), 1415–1424 (2012).

- **This exhaustive lipid metabolite profiling study is a powerful basis for the development of biomarkers that are representative the disease activity characteristic of RA.**

41 Borgeat P, Fruteau De Laclos B, Picard S, Drapeau J, Vallerand P, Corey EJ. Studies on the mechanism of formation of the 5S, 12S-dihydroxy-6,8,10,14(E,Z,E,Z)-icosatetraenoic acid in leukocytes. *Prostaglandins* 23(5), 713–724 (1982).

42 Marcus AJ, Broekman MJ, Safier LB *et al.* Formation of leukotrienes and other hydroxy acids during platelet-neutrophil interactions *in vitro*. *Biochem. Biophys. Res. Commun.* 109(1), 130–137 (1982).

43 Jónasdóttir HS, Nicolardi S, Jonker W *et al.* Detection and structural elucidation of esterified oxylipids in human synovial fluid by electrospray ionization-fourier transform ion-cyclotron mass spectrometry and liquid chromatography-ion trap-MS3: detection of esterified hydroxylated docosapentaenoic acid containing phospholipids. *Anal. Chem.* 85(12), 6003–6010 (2013).

44 Weljie AM, Dowlatabadi R, Miller BJ, Vogel HJ, Jirik FR. An inflammatory arthritis-associated metabolite biomarker pattern revealed by 1H NMR spectroscopy. *J. Proteome Res.* 6(9), 3456–3464 (2007).

- **Identified, for the first time, metabolites from the nucleic acid and oxidative stress pathways as potential biomarkers for RA pathology.**

45 Lauridsen MB, Bliddal H, Christensen R *et al.* 1H NMR spectroscopy-based interventional metabolic phenotyping: a cohort study of rheumatoid arthritis patients. *J. Proteome Res.* 9(9), 4545–4553 (2010).

46 Dursunoğlu D, Evrengül H, Polat B *et al.* Lp(a) lipoprotein and lipids in patients with rheumatoid arthritis: serum levels and relationship to inflammation. *Rheumatol. Int.* 25(4), 241–245 (2005).

47 Georgiadis A, Papavasiliou E, Lourida E *et al.* Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment – a prospective, controlled study. *Arthr. Res. Ther.* 8(3), R82 (2006).

48 Madsen RK, Lundstedt T, Gabrielsson J *et al.* Diagnostic properties of metabolic perturbations in rheumatoid arthritis. *Arthr. Res Ther.* 13(1), R19 (2011).

49 Gerber DA. Low free serum histidine concentration in rheumatoid arthritis. A measure of disease activity. *J. Clin. Invest.* 55(6), 1164–1173 (1975).

50 Liao KP, Cai T, Gainer VS *et al.* Lipid and lipoprotein levels and trend in rheumatoid arthritis compared to the general population. *Arthr. Care Res.* 65(12), 2046–2050 (2013).

51 Jiang M, Chen T, Feng H *et al.* Serum metabolic signatures of four types of human arthritis. *J. Proteome Res.* 12(8), 3769–3779 (2013).

- **Results of this study indicate a common core of metabolic pathways associated with arthritis, which are explained by common pathogenic mechanisms such as hypoxia.**

52 Young SP, Kapoor SR, Viant MR *et al.* The impact of inflammation on metabolomic profiles in patients with arthritis. *Arthr. Rheum.* 65(8), 2015–2023 (2013).

53 Madsen R, Rantapää-Dahlqvist S, Lundstedt T, Moritz T, Trygg J. Metabolic responses to change in disease activity during tumor necrosis factor inhibition in patients with rheumatoid arthritis. *J. Proteome Res.* 11(7), 3796–3804 (2012).

54 Van Der Pouw Kraan TCTM, Wijbrandts CA, Van Baarsen LGM *et al.* Rheumatoid arthritis subtypes identified by genomic profiling of peripheral blood cells: assignment of a type I interferon signature in a subpopulation of patients. *Ann. Rheum. Dis.* 66(8), 1008–1014 (2007).

55 Stephens NS, Siffledeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. *J. Crohn's Col.* 7(2), e42–e48 (2013).

56 Kapoor SR, Filer A, Fitzpatrick MA *et al.* Metabolic profiling predicts response to anti-tumor necrosis factor alpha therapy in patients with rheumatoid arthritis. *Arthr. Rheum.* 65(6), 1448–1456 (2013).

- **Demonstrates the utility of urine to develop biomarkers for personalized treatment in psoriatic arthritis and RA.**

57 Sitton NG, Dixon JS, Bird HA, Wright V. Serum biochemistry in rheumatoid arthritis, seronegative arthropathies, osteoarthritis, SLE and normal subjects. *Rheumatology* 26(2), 131–135 (1987).

58 Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus* 15(5), 308–318 (2006).

59 Ouyang X, Dai Y, Wen JL, Wang LX. 1H NMR-based metabolomic study of metabolic profiling for systemic lupus erythematosus. *Lupus* 20(13), 1411–1420 (2011).

- **Commonly altered lipid profile in systemic lupus erythematosus and RA could explain the increased incidence of cardiovascular disease observed for both rheumatic diseases.**

60 Watanabe M, Suliman ME, Qureshi AR *et al.* Consequences of low plasma histidine in chronic kidney disease patients: associations with inflammation, oxidative stress, and mortality. *Am. J. Clin. Nutr.* 87(6), 1860–1866 (2008).

61 De Carvalho JF, Bonfá E, Borba EF. Systemic lupus erythematosus and "lupus dyslipoproteinemia". *Autoimmun. Rev.* 7(3), 246–250 (2008).

62 Xinghong D, Ying Y. Serum metabonomics study of systemic lupus erythematosus by rapid resolution liquid chromatography coupled with Q-TOF mass spectrometry. Presented at: *Biomedical Engineering and Biotechnology (iCBEB), 2012 International Conference*. Macau, China, 28–30 May 2012.

63 Wu T, Xie C, Han J *et al.* Metabolic disturbances associated with systemic lupus erythematosus. *PLoS ONE* 7(6), e37210 (2012).

64 Li Y, Tucci M, Narain S *et al.* Urinary biomarkers in lupus nephritis. *Autoimmun. Rev.* 5(6), 383–388 (2006).

65 Mosley K, Tam FWK, Edwards RJ, Crozier J, Pusey CD, Lightstone L. Urinary proteomic profiles distinguish between active and inactive lupus nephritis. *Rheumatology* 45(12), 1497–1504 (2006).

66 Oates JC, Varghese S, Bland AM *et al.* Prediction of urinary protein markers in lupus nephritis. *Kidney Int.* 68(6), 2588–2592 (2005).

67 Zhang X, Jin M, Wu H *et al.* Biomarkers of lupus nephritis determined by serial urine proteomics. *Kidney Int.* 74(6), 799–807 (2008).

68 Romick-Rosendale L, Brunner H, Bennett M *et al.* Identification of urinary metabolites that distinguish membranous lupus nephritis from proliferative lupus nephritis and focal segmental glomerulosclerosis. *Arthr. Res. Ther.* 13(6), R199 (2011).

69 Christians U, Schmitz V, Schoning W, Bendrick-Peart J, Klawitter J, Haschke M. Toxicodynamic therapeutic drug monitoring of immunosuppressants: promises, reality, and challenges. *Ther. Drug Monit.* 30(2), 151–158 (2008).

70 Brey RL, Holliday SL, Saklad AR *et al.* Neuropsychiatric syndromes in lupus: prevalence using standardized definitions. *Neurology* 58(8), 1214–1220 (2002).

71 Alexander JJ, Zwingmann C, Quigg R. MRL/lpr mice have alterations in brain metabolism as shown with [1H–13C] NMR spectroscopy. *Neurochem. Intern.* 47(1–2), 143–151 (2005).

72 Kaiser E, Chiba P, Zaky K. Phospholipases in biology and medicine. *Clin. Biochem.* 23(5), 349–370 (1990).

73 Korotkova M, Jakobsson P-J. Persisting eicosanoid pathways in rheumatic diseases. *Nat. Rev. Rheumatol.* 10(4), 229–241 (2014).

74 Braun J, Sieper J. Ankylosing spondylitis. *Lancet* 369(9570), 1379–1390

75 Fischer R, Trudgian DC, Wright C *et al.* Discovery of candidate serum proteomic and metabolomic biomarkers in ankylosing spondylitis. *Mol. Cell Proteom.* 11(2), M111.013904 (2012).

• **Shows the utility of the plasmatic metabolome to differentiate ankylosing spondylitis patients from controls as well ankylosing spondylitis patients according to disease activity.**

76 Gao P, Lu C, Zhang F *et al.* Integrated GC-MS and LC-MS plasma metabonomics analysis of ankylosing spondylitis. *Analyst* 133(9), 1214–1220 (2008).

77 Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat. Clin. Pract. Endocrinol. Metab.* 4(2), 80–90 (2008).

78 Julià A, Tortosa R, Hernanz JM *et al.* Risk variants for psoriasis vulgaris in a large case–control collection and association with clinical subphenotypes. *Hum. Mol. Genet.* 21(20), 4549–4557 (2012).

79 Arden N, Nevitt MC. Osteoarthritis: epidemiology. *Best Pract. Res. Clin. Rheumatol.* 20(1), 3–25 (2006).

80 Lamers RJ, Degroot J, Spies-Faber EJ *et al.* Identification of disease- and nutrient-related metabolic fingerprints in osteoarthritic guinea pigs. *J. Nutr.* 133(6), 1776–1780 (2003).

81 Malone DG, Irani AM, Schwartz LB, Barrett KE, Metcalfe DD. Mast cell numbers and histamine levels in synovial fluids from patients with diverse arthritides. *Arthr. Rheum.* 29(8), 956–963 (1986).

82 Tetlow LC, Woolley DE. Histamine stimulates the proliferation of human articular chondrocytes *in vitro* and is expressed by chondrocytes in osteoarthritic cartilage. *Ann. Rheum. Dis.* 62(10), 991–994 (2003).

83 Zhai G, Wang-Sattler R, Hart DJ *et al.* Serum branched-chain amino acid to histidine ratio: a novel metabolomic biomarker of knee osteoarthritis. *Ann. Rheum. Dis.* 69(6), 1227–1231 (2010).

•• **Uses a well-structured study design including a discovery phase and an independent validation phase to validate the metabolite associations with osteoarthritis.**

84 Felig P, Marliss E, Cahill GF Jr. Plasma amino acid levels and insulin secretion in obesity. *N. Engl. J. Med.* 281(15), 811–816 (1969).

85 Caballero B, Gleason RE, Wurtman RJ. Plasma amino acid concentrations in healthy elderly men and women. *Am. J. Clin. Nutr.* 53(5), 1249–1252 (1991).

86 Adams Jr SB, Setton LA, Kensicki E, Bolognesi MP, Toth AP, Nettles DL. Global metabolic profiling of human osteoarthritic synovium. *Osteoarthr. Cartilage* 20(1), 64–67 (2012).

87 Doherty M. New insights into the epidemiology of gout. *Rheumatology* 48(Suppl. 2), ii2–ii8 (2009).

88 Liu Y, Sun X, Di D, Quan J, Zhang J, Yang X. A metabolic profiling analysis of symptomatic gout in human serum and urine using high performance liquid chromatography-diode array detector technique. *Clin. Chim. Acta* 412(23–24), 2132–2140 (2011).

89 Cui Q, Lewis IA, Hegeman AD *et al.* Metabolite identification via the Madison Metabolomics Consortium Database. *Nat. Biotech.* 26(2), 162–164 (2008).

90 Tautenhahn R, Cho K, Uritboonthai W, Zhu Z, Patti GJ, Siuzdak G. An accelerated workflow for untargeted metabolomics using the METLIN database. *Nat. Biotech.* 30(9), 826–828 (2012).