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LIQUID BIOPSIES TO GUIDE THERAPEUTIC DECISIONS IN RHEUMATOID ARTHRITIS

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Abstract

Rheumatoid arthritis (RA) is a systemic, immune-mediated inflammatory disease that has transitioned from a debilitating disease to a chronic, controllable disease. This has been possible due to the introduction of new treatment strategies like “treat-to-target”, in which the clinician treats the patient aggressively enough to reach low disease activity or remission, and the introduction of new therapeutic agents, such as biological therapies, which can lead to the prevention of damage by early diagnosis and initiation of treatment. Attention is now being directed towards identifying the optimal treatment for each patient, one that will be the most efficient and have the least number of side effects. Much work has been done to find serological and synovial biomarkers of response to various RA treatments. Proteomics, genomics and, in the past few years, metabolomics, have all been used in the quest of identifying these biomarkers. Blood-based liquid biopsies provide a minimally invasive alternative to synovial biopsies to identify cellular and molecular signatures that can be used to longitudinally monitor response and allow for personalized medicine approach. Liquid biopsies are comprised of cell free DNA, immune circulating cells, and extracellular vesicles, and are being increasingly and successfully used in the field of oncology for diagnosis, progression, prognosis, and prediction of response to treatment. Recently, researchers have also begun investigating the usefulness of liquid biopsies in the field of rheumatology; in this review we will focus on the potential of liquid biopsy blood samples as biomarkers of response to treatment in patients with RA.

Keywords

rheumatoid arthritis; liquid biopsies; synovial biopsies; biomarkers

Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis, affecting between 0.5 to 1 % of the population worldwide (1). Though it was originally considered benign, it has

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since been proven to be debilitating, and patients with RA have an increased death rate when compared to the general population (2). Since 1898, when aspirin was introduced as a treatment for rheumatic fever, there have been great changes in RA treatment due to a better understanding of its pathogenesis (3).

In the first half of the 20th century, a pyramidal model was used to treat RA: in the first stage, only symptomatic treatments were used; these included salicylates, from which non-steroidal anti-inflammatory drugs (NSAIDs) are derived, and analgesics. This stage was associated with bed rest, splinting, physical therapy, heat therapy, and occupational therapy. The second stage saw the addition of disease-modifying disease drugs (DMARDs) such as gold salts, methotrexate, and penicillamine (Figure 1). At the time, this method of treatment was justified based on the assumption that DMARDs were too toxic for routine use. (4).

A few significant developments in the understanding of RA contributed to a shift in treatment towards DMARDs. Firstly, RA became widely acknowledged as a seriously debilitating disease; Wolfe and Sharpe clearly demonstrated that patients with untreated RA developed severe joint damage due to increasing deformities, progression of erosions, and joint space narrowing (8). Secondly, it was shown that NSAIDs were not benign, as had been previously thought, and had associated gastrointestinal complications (9–12). Often, patients who were hospitalized or died due to NSAID complications tended to be older, had longer disease duration, higher disability scores, and higher incidence of comorbidities (10). Thirdly, there was accumulating evidence that DMARDs were efficient in controlling symptoms, interfering with joint destruction, and decreasing inflammation in RA. Consequently, DMARDs were shown to provide better control in disease progression, improvement in pain symptoms, and less disability than other treatments (13,14). There was also growing evidence that DMARDs are no more toxic than NSAIDs, making them an increasingly practical option for RA treatment (13). Finally, a number of new therapeutic agents were made available to the medical community, including pharmacological and biologic-(b)DMARDs (15–17).

These developments have allowed for new approaches to RA treatment (Figure 1). DMARDs and bDMARDs are being used earlier in treatment and are continued throughout the course of the disease with the aim of maintaining the patient's level of disability close to normal (5, 6). Since the initial approval of tumour necrosis factor (TNF)-neutralizing therapies, other biologic agents have been approved, such as the anti-T cell (and/or dendritic cell) therapy, the B cell-depleting antibody, an interleukin (IL)-6 receptor antagonist, and drugs targeting IL-23 and IL-17 cytokines. In addition, a janus-kinase inhibitor (JAK) has recently been added into the RA therapeutic armamentarium (7). Because choosing and initiating the right treatment earlier in the course of disease could help the patient reach remission, considerable efforts are being made to develop the tools necessary to employ a 'personalized' medicine approach. This approach can potentially allow physicians to match patients with the most appropriate therapy option for their disease subtype.

Identification of biomarkers for diagnosis, prognosis, monitoring of therapeutic responses and selection of patients for specific DMARDs therapies is urgently required, and is therefore an area of intensive research. For this purpose, researchers have used synovial

tissue, obtained from the joints by arthroscopy, surgery or ultrasound-guided biopsy, synovial fluid obtained during surgery, or more commonly by arthrocentesis, and peripheral blood. Obtaining synovial tissue or synovial fluid is inherently more risky to the patient than taking a blood sample. In addition, taking further repetitive synovial tissue or fluid samples during treatment is difficult. More easily accessible samples, such as blood, are preferred for researchers, as they involve less risk for patients and less hassle for scientists. Blood samples allow quantification of different types of biomarkers using proteomics, lipidomics, transcriptomics, or RNA sequencing, and the processing of samples for these methods is relatively easy (8). Thus, blood-based liquid biopsies provide a minimally invasive alternative to standard synovial biopsies in order to identify cellular and molecular signatures that can be used to longitudinally monitor response and allow for a personalized medicine approach. In this review we will focus on the potential of using the blood-based liquid biopsy to study biomarkers of response to treatment in patients with RA.

Synovial biopsy

Liquid biopsies intend to get information about the main tissues involved in a specific disease pathogenesis (9). Therefore, we will start with a brief review of the advances in synovial biopsy studies for a personalized medicine approach in RA (10). Yet, although the synovial tissue is the main target of inflammation in RA (11), recent work has shown that systemic autoimmunity precedes synovial inflammation in RA, with several altered lymphoid cell subsets in lymph node biopsies obtained during earlier phases of RA (12–14). Furthermore, animal models have suggested that lymph node changes may precede inflammation in the synovial tissue (15).

The rheumatoid synovium presents with a hyperplastic intimal lining layer and with accumulation of recruited inflammatory cells in the sub-lining (16). An increasing number of studies have focused on studying synovial tissue in order to increase knowledge of disease pathogenesis, explore early diagnosis and biomarkers for disease activity, and evaluate response to treatment (17–22). Advances in synovial biopsies such as arthroscopy and ultrasound-guided biopsy have increased accessibility of synovial samples, allowing for such research (23). While a number of studies focus on identifying features of the inflamed synovium to allow for early diagnosis of the disease, a greater number are focused on finding markers that can predict response to different types of treatment, allowing the clinician to orient his practice towards personalized medicine. Many of the biomarkers discovered for this purpose are cellular markers identified by immunohistochemistry, synovial cytokines, chemokines, and gene-expression profiles.

Lymphocyte aggregates.

Though these are only found in about 30% of RA patients and there is no correlation with disease activity (24, 25), one study found that the number of lymphocyte aggregates was predictive of clinical response to infliximab (26).

Lymphocytes.

It has long been shown that RA presents with an increase in the number of cells infiltrating the synovium (27, 28). Cells in the synovium are studied by immunostaining and cell counting. Numerous studies have described a decrease in the number of T cells after treatment with prednisolone (CD4, CD5 and CD38 plasma cells) (29), conventional synthetic disease-modifying drugs (csDMARDs) like methotrexate (CD3, CD8 and CD38 plasma cells) (30) and bDMARDs like infliximab (CD3⁺ cells (31) and CD22⁺ cells (32)) and rituximab (B cells, T cells and macrophages) (33–36).

Macrophages.

Macrophages (CD68⁺ cells) appear to be the most convincing cellular biomarker of response to treatment. This has been demonstrated by several studies and is independent of treatment type (36, 37). Studies have shown that prednisolone (29), methotrexate (38), leflunomide (39) and infliximab (32, 40), produced a decrease in the number of macrophages in the synovial sub-lining. Anakinra (41), rituximab, and methotrexate reduced the number of macrophages in the intimal layer, and gold salts did so in all synovial layers. Importantly, the decrease in the number of CD68⁺ cells has been shown to correlate with clinical improvement (36, 37). For this reason, CD68⁺ has been proposed as a biomarker of response for new therapies (36).

Fibroblast like synoviocytes(FLS).

FLS are synovial resident cells that express characteristic markers on their surface including intercellular adhesion molecule 1 (ICAM-1), podoplanin (PDPN), vascular adhesion molecule 1 (VCAM-1), and CD55; these markers are used for FLS identification via immunohistochemistry (42). Few papers have studied FLS in the synovial membrane, their phenotyping, and their response after treatment. However, a recent paper gave comprehensive epigenomic descriptions of RA FLS (43), and another has identified different FLS subpopulations by RNA-seq analysis (44), with different synovial localization and gene expression signatures. Still, further experimentation is needed to correlate these new findings with patient stratification and therapeutic response.

Cytokines and chemokines.

Several synovial proinflammatory cytokines have been studied as potential biomarkers, including TNF α , IL-6 and IL-1 β . Although the pretreatment levels of these cytokines correlates with disease activity (45), there is scarce and contradictory information on their value as possible biomarkers of response to targeted therapy (30, 39, 46). Two groups found that RA patients who responded to TNF inhibitors had higher TNF- α levels in the synovial tissue (determined by immunostaining) (40, 45). One group found that synovial phosphorylated STAT is decreased after treatment with Tofacitinib, indicating that cytokine-related signaling could also potentially be used to predict response to treatment. (47). Finally, patients who responded to infliximab treatment also showed decreased monocyte chemotactic protein 1 (MCP1) and IL-8 (32).

Gene expression studies

In a small study of 18 RA patients with active disease, one group found differences in the gene expression profiles between responders versus non-responders to infliximab. Interestingly, the genes related to inflammation were upregulated in responders (48). Another group found that RA patients who had good response to adalimumab also had different gene expression profiles than non-responders, showing an overexpression of genes related to regulation of immunity and cell division. Patients that were poor responders to treatment had higher baseline levels of IL-18, IL-18 receptor accessory, IL-7 receptor α chain and proliferation marker Ki-67 (49). Promising results were obtained by Dennis (50) et al that described 4 groups of synovial subtypes using array analysis: myeloid, lymphoid, low inflammation and fibroid subtypes. They showed that patients with an overexpression of the myeloid signature and TNF- related genes have a better response to TNF inhibitor; these results were confirmed in another independent study (51). Parting from the 4 subtypes of synovial signature, the authors went further to find possible serum biomarkers that could be representative of different synovial subtypes. They found that a combination of 2 biomarkers could predict response to tocilizumab and adalimumab: soluble intercellular adhesion molecule (sICAM) and C-X-C motif chemokine 13 (CXCL13).

Liquid biopsy

While several authors continued their work to identify biomarkers that predict response to therapy in synovial tissue, other groups have considered the possibility that liquid biopsies, which are already being used in oncology, could lead to more predictive biomarkers. The liquid biopsy is an emerging and promising detection tool that is both non-invasive and convenient (52). Liquid biopsies can be used to analyze tissue-derived information, including circulating cell free DNA (cfDNA), circulating tissue-derived cells, and exosomes in the blood or other bodily fluids (53). Several studies suggest that liquid biopsies are useful to guide therapeutic decisions in cancer (54–56), although less information is available on synovial-derived information and the role of liquid biopsies in autoimmune diseases including RA (Figure 2).

A. Cell free DNA

Cell free DNA (cfDNA) was first detected in human blood in 1948 (57); in 1977, it was discovered that patients with cancer had higher levels of cfDNA (58) than the general population. Moreover, patients with metastasis had higher levels of cfDNA than patients without, and these levels decreased with therapy (58). Thus, cfDNA detection is mainly used in cancer patients for disease monitoring and response to treatment. Of interest, high levels of cfDNA were also found in several other conditions such as trauma, infections, exercise, transplantation, cerebral infarctions, and inflammation (59). It is thought that cfDNA is released from cells by at least two nonexclusive mechanisms: a passive one and an active one.

The passive mechanism is the process by which apoptotic and necrotic cells release nuclear and mitochondrial DNA when they die. In physiological conditions, macrophages eliminate the fragments that result from cell death, but in cancer and inflammation there are alterations

to this process, increasing the amount cfDNA in the plasma (60, 61). The active mechanism involves the spontaneous release of DNA fragments by cells in circulation, which might also increase due to inflammation, and has been observed in cultured cells of different origins (62–64). However, the exact mechanism of cfDNA release remains elusive. Other sources could include release of DNA to form neutrophil extracellular traps (NETs)(65); this could be another source of cfDNA in plasma of RA patients, as RA-derived neutrophils are more prone to NETosis (66, 67).

In contrast to the growing amount of information on cfDNA in cancer, literature on this topic in autoimmune disease is scarce. However, the literature that can be found is promising, and cfDNA appears to be a good candidate as a biomarker of early diagnosis of RA (68), for disease monitoring (69) and prediction of response to treatment (70). In the 1970s, it was shown that cfDNA levels were higher in the plasma of RA patients with less than 10 years of disease evolution in comparison to healthy controls, especially in seronegative patients with more severe disease (71, 72). Other studies corroborated this finding (73), (69, 70). Of note, cfDNA levels were in a high range, similar to ones reported in neoplasia (74). Studies also evaluated the effect of different therapies on cfDNA levels. CfDNA concentrations in plasma and serum did not vary with the administration of classical DMARDs such as methotrexate, hydroxychloroquine, or sulfasalazine, or in-between doses of TNF inhibitors. However, after the infusion of anti-TNF monoclonal antibody, cfDNA significantly changed in 70% of the patients, suggesting that DNA release could be a TNF-related mechanism (73).

Another study evaluated not only nuclear but also mitochondrial DNA, both cell free and cell-surface bound (csb) (69). In this study, only csb mitochondrial DNA was found to be a good discriminant between RA patients and controls. The authors showed that cfDNA concentrations were higher in RA patients with higher disease activity (Disease activity Score (DAS28) > 5.1) in comparison to patients with lower disease activity and healthy controls. This study also included a group of 14 patients that received rituximab and achieved EULAR (European League Against Rheumatism) moderate/good response at week 24. They found that csb mitochondrial DNA levels tended to be lower in patients treated with rituximab in comparison to the group that received only methotrexate (69). Another study (70) suggests that levels of cfDNA might decrease after long periods of low disease activity or with disease remission. Of note, an increase in cfDNA was observed 8 weeks post-treatment initiation, which correlated with the improvement of the Simple Clinical Disease Activity Index(SDAI), which could be related to the apoptosis in the synovium observed after anti-TNF therapy. Finally, Kawane et al. observed that DNase II knockout mice developed symmetric polyarthritis, and suggested that cfDNA (75))might be directly implicated in the etiology of RA. Unfortunately, these studies did not explore whether a lack of DNase II in mice is associated with higher levels of cfDNA.

Recently, two groups noticed that epigenomic (and thus cell-type specific) information can be detected by examining cfDNA (76, 77). These studies are based on the rationale that cfDNA contains an epigenomic footprint that can be used to identify the origin of a dying tissue. Genomic DNA is fragmented by enzymes that prefer to cut in unexposed sections that aren't protected by nucleosomes. Therefore, the cfDNA fragments released are those protected by nucleosomes and transcription factors. Based on this rationale, one group

examined the nucleosome positioning of cfDNA (76). By identifying known nucleosome positioning profiles, these patterns can be used to identify cell type of origin. Another group investigated whether or not cfDNA methylation could also be a marker of tissue type (77). The team profiled a number of tissues using a methylation array to find tissue-specific CpGs (5'—C—phosphate—G—3'). They then examined cfDNA in the blood and identified tissue-specific patterns of methylation. Future studies will be needed to find out whether or not this information can be helpful to match cfDNA to specific synovial tissue.

B. Circulating immune cell profiling in rheumatoid arthritis

RA is a systemic autoimmune disease, and previous work has suggested that lymph node changes may precede inflammation in the synovial tissue (12–14). Importantly, several studies have also shown expanded peripheral cell subsets that are recruited into the RA joint (78), suggesting that peripheral blood is a good source for immune cell profiling. On the other hand, there is no evidence that resident synoviocytes (fibroblast-like synoviocytes and macrophages-like synoviocytes) can migrate outside the joint and circulate peripherally, despite the FLS well-known ability to invade and migrate in vitro (79, 80), and animal models suggesting the possibility of circulating FLS (81). In this review, we will therefore consider circulating immune cells as liquid biopsies, as they might originate in other pathogenic organs in RA patients, such as the lymph nodes, and have the potential to give information on the patient's disease.

In RA, the cells that drive immune responses are also druggable targets (B and T cell, macrophage, FLS), making them promising biomarker candidates. Moreover, immune cells might not only provide information about the *status quo* of an immune response, but also about their histories (for example, through measurement of the frequency and specificity of memory T cells) and potentially about their futures (for example, by measuring cellular responses to *in vitro* stimulation). Several assays, including flow cytometry, mass cytometry, gene expression profiling by RNA-sequencing, and cellular function assays, have been shown to be helpful in the analysis of circulating cells in RA patients (82).

1. Gene expression of immune circulating cells—Gene expression profiling is used to analyze expression of thousands of genes in order to create a global picture of biological functions for the population of interest. (83). Gene expression profiling is at the forefront of personalized medicine, especially in oncology (84). Because inflammation is a biological process that involves several cell types and produces many inflammatory factors, changes in gene expression could provide information about activated signatures or activated cell populations at different stages of RA.

Although many groups have studied gene expression in immune cells as biomarkers, the results have not been very encouraging. The only exception is the prediction of response to rituximab (85, 86). Two groups showed that a type-I interferon (IFN) signature in whole blood was a predicting biomarker of response. Good responders had a low or absent IFN response activity at baseline, whereas non-responders displayed an activated type-I IFN system before the start of treatment. A set of 305 IFN type-I genes were validated as a predictor of non-response to rituximab. These results suggest that RA patients with a high

IFN signature represent a different pathogenic subset of patients. Another study (87) observed an increase in IFN response activity in patients who responded to rituximab therapy, while the IFN signature in non-responders stayed the same. In responders, the IFN signature score returned to baseline values 6 months after the start of treatment. Future studies are necessary to determine whether this phenomenon shows that an increase in IFN response activity during rituximab treatment is necessary for good response or is merely a non-causal correlation.

2. Phenotypic characterization of immune cell subsets—Cellular

immunophenotyping by flow cytometry (which can detect as many as 20 parameters per cell) and mass cytometry (which can measure more than 50 parameters per cell) allows for analysis of cell surface markers and analysis of cytoplasmic and nuclear proteins, including cytokines, cell proliferation markers, cell signaling responses, and transcription factors. In RA, several studies have analyzed immune cell subsets in peripheral blood, and showed expansion of several subsets including IL-17-producing helper T cells relative to regulatory T cells (88, 89), terminally differentiated, cytotoxic CD4⁺CD28⁻ T cells (90), CD4⁺ T cells that were positive for activation-induced surface markers (91), immature cells characterized as CD45RB(bright)CD45RA(+)CD62L(-), a large population that coexpresses CD45RA and CD45RO (92), and recently, PD-1^{hi}CXCR5⁻ 'peripheral helper' T (T_{PH}) cells that also express factors enabling B-cell help, including IL-21, CXCL13, ICOS, and MAF (78).

Still, only a small number of studies have reported an association of immune cell populations to response to certain treatment. For instance, high frequency of CD4⁺CD28⁻ T cells was associated with a poor clinical response prior to initiation of anti-TNF therapy (93). An interesting dichotomy was described with cCD27⁺ memory B cells. While low numbers of cCD27⁺ memory B cells and CD27^{hi}CD38^{hi} pre-plasma circulating cells at baseline were associated with a better clinical response to rituximab (94, 95), a high frequency of CD27⁺ memory B cells was correlated with a favorable response to TNF inhibition (96). Finally, the analysis of CD19⁺ cells in the peripheral blood from RA patients who are treated with B-cell therapy (rituximab) is the only routine application of flow cytometry in current rheumatology practice and is used to monitor B-cells. Some studies suggest that rituximab dosage should be adjusted based on the number of residual circulating B cells after rituximab therapy (97).

3. In vitro assays of immune circulating cells—

Several *in vitro* assays can be used to assess immune cell function (cell proliferation, changes in gene or protein expression, or phagocytosis). Still, functional cellular assays are not present as routine clinical laboratory assays. A possible immunophenotyping approach would be to trigger polyclonal rheumatic populations with different ligands and then measure the secretion of several analytes to assess induced innate or adaptive immune responses (98). This approach could potentially identify dominant pathways in individual patients and provide guidance for therapeutic decisions in rheumatoid arthritis.

C. Extracellular vesicles

Extracellular vesicles (ExV) are particles released from cells that are found in all types of bodily fluids. They can originate in endosomes (exosomes) or bud directly from the cell membrane (microvesicles). In this review we will be referring to them as ExV. These vesicles contain or expose a variety of molecules on their surfaces, ranging from lipids, RNA, proteins, and DNA; these molecules can be useful for exosome identification. It was shown that ExV have different content amongst them, depending on their parental cells, and have a role in cell to cell communication. They can cause changes in gene transcription, including genes involved in inflammation (99, 100) and cell proliferation (101–103). In cancer, they can contribute to disease progression by participating in the crosstalk between tumor cells and stromal cells and can aid in metastasis by carrying behavior-changing information to cells located at a distance from the primary tumor (104).

Disease conditions change the specific content and membrane composition of ExV. Thus, these EVs might serve as biomarkers of disease. In RA, the ExV originate from a multitude of cells that infiltrate the synovial membrane (Figure 2). It was demonstrated that in patients with RA, the total number of ExV was significantly higher in comparison to healthy controls (105). In addition to qualitative differences, quantitative differences were also found between RA patients and healthy controls. One study found 10 proteins specific for extracellular vesicles in RA patients. Interestingly, more than half of the proteins were citrullinated (106).

Changes in ExV content (lipids, proteins, DNA, and miRNA) could be used as potential biomarkers for different stages of disease (107–110). MiRNAs are fragments of single stranded, non-coding RNA, comprised of 19–25 nucleotides (111). There is strong accumulating evidence for the potential use of miRNAs as biomarkers of diagnosis, disease progression, and prediction of response to treatment, resulting in increased exploration of miRNAs in various diseases. Although most of these studies do not specify the location of miRNAs, it was shown that most plasma miRNA is contained in ExVs (112).

In rheumatology, an increasing number of studies are being done on both the role of miRNA in the pathogenesis of RA and on its potential as a biomarker. Several studies have shown that the expression of miRNAs is altered in immune and resident cells that are involved in the pathogenesis of RA, and contributes to the typical features of RA (113); these studies have also shown that miRNA's could potentially be therapeutic predictive biomarkers. For instance, higher pretreatment levels of miR-16 in the sera of treatment naïve patients with early RA was predictive of better improvement in disease activity during the first 3 months of follow-up, after therapy was initiated with conventional DMARDs (114). In a study by Castro-Villega et al (115), investigators measured plasma miRNA in 95 patients with active RA before treatment and 6 months after initiation of a combined therapy with anti-TNF/DMARDs. They found 75 miRNAs that were upregulated and 9 that were downregulated after treatment. In general, the miRNAs were downregulated in patients that were non-responders to treatment versus responders. Furthermore, a negative correlation was found between the changes in the expression levels of almost all of the validated miRNAs and the changes in various clinical and inflammatory parameters. ROC analyses demonstrated that

high levels of hsa-miR-23-3p and hsa-miR-223-3p might act as predictors of response to therapy.

Another study (116), a placebo-controlled, double-blind, prospective study of patients with early RA, found an association between whole blood miR-22 and miR-886.3p, and response to adalimumab. Specifically, the probability for achieving a good response to adalimumab (in combination with methotrexate) increased from 65% to 95% in patients with high expression of miR-886.3p and low expression of miR-22. Cuppen et al (117) tried to reproduce the results of some of the previous studies using a discovery cohort and then a validation cohort. Higher circulating values of miR23a were found to be predictive of response to TNF inhibitors and showed similar results to a study by Krintel et al (116). However, some of their results were contradictory to previous reports. A recently published study by Sode et al (118), on a cohort of patients with early RA treated with a combination of adalimumab, methotrexate, and glucocorticoids found that a higher pretreatment level of miR-27a-3p and a decrease in miR-27a-3p level during the first 3 months was associated with ACR/EULAR Boolean remission at 12 months. Finally, one study (119) showed that miR-125b was higher in RA patients in comparison to healthy controls and that its baseline level was negatively correlated with disease activity. The levels increased after 3 months of therapy and the higher baseline cellular levels were predictive of a better response to DMARDs. Higher levels of miR-125 b were also found to be predictive of better response to rituximab (120).

Conclusions and future directions

Despite a great number of studies in the fields of epigenetics, genetics, and proteomics, no biomarker for prediction of response to RA treatment has yet been identified. Reasons for this include a lack of standardized protocols for the identification of different candidate biomarkers and, sadly, the lack of validation of existing results. Over the last few years investigators have started to look into other options for biomarker exploration including metabolomics, synovial biopsies, and liquid biopsies; these have potential to guide therapeutic decisions in RA. Although few metabolomic studies have been conducted so far, our group showed that a serum metabolomic profile identified by H-NMR and UPLC-MS/MS could be predictive of response to rituximab (121). Of note, our unpublished results also suggest that serum metabolomics profiles might predict synovial gene expression, (Figure 3) suggesting that metabolomics may be a promising tool for predicting specific pathogenic pathways in the inflamed synovium of RA patients. With the advent of ultrasound-guided synovial biopsy, examination of pathobiological specimens from the target organ, the arthritic synovium, might become a part of standard clinical care and help with management decisions, much like other branches of medicine including gastroenterology, dermatology and nephrology. Finally, the practice of liquid biopsies has revolutionized the care of patients with cancer. In RA, though liquid biopsies are still in their infancy, technical advances and some encouraging clinical studies suggest that they hold great promise in the near future.

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Figure 1. Historic pyramidal treatment approach and its current inverted aspect.

A) Previously, NSAIDs made up the base of RA treatment followed by DMARDs and experimental therapies. DMARDs were added later in the course of disease, as they were considered more toxic than NSAIDs or prednisone. Surgery and physical therapy were also important, and intraarticular corticosteroids were administered during flares. B) Over time, there has been an inversion of the original pyramid, and DMARDs are now introduced early in the course of disease followed by biological therapies if control of the disease is not reached. The goal is to maintain a minimum disease activity, or remission if possible, on the least number of medications. Corticosteroids are still being used for arthritic flares, but surgery is rarely necessary because damage is generally prevented by early treatment.

Figure 2. Cells involved in RA pathogenesis and components of liquid biopsies

A) This figure shows a synovial joint which is normal on the left and affected by rheumatoid arthritis on the right. The left side depicts a normal synovial lining while the right side displays a hyperplastic synovial lining. At the same time an erosion is also present on the affected side of the joint. The diseased side of the joint shows an accumulation of various types of cell that are involved in the pathogenesis of RA including fibroblast-like synoviocytes, B and T cells, macrophages, neutrophils, plasma cells and dendritic cells. B) Circulating free DNA (cfDNA), circulating immune cells (CIC), and exosomes containing for instance microRNA can be used to analyze synovium-derived information.

Figure 3. Serum metabolites correlation with synovial CD3E and CD19.

Nineteen active seropositive RA patients on concomitant methotrexate were studied. One of the involved joints was a knee or a wrist appropriate for arthroscopy. A Bruker Avance 700 MHz spectrometer was used to acquire NMR spectra of serum samples. Gene expression in synovial tissue obtained by arthroscopy was analyzed by real-time PCR. Correlation of serum metabolites for CD3E and CD19 using linear regression, controlling for both age and gender was conducted. Overview of the metabolites identified by NMR was organized by metabolic pathway. Metabolites that were negatively correlated with CD3E and CD19 are shown in red. Abbreviations: TMA: trimethylamine, TMAO: trimethylamine N-oxide, DMA: NN-dimethylamine, THF: Tetrahydrofolate, IMP: inosine monophosphate.