Biomarkers in Clinical Trials for Rheumatoid Arthritis

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1 Introduction

Rheumatoid arthritis (RA) is a chronic multisystem disease that is characterized by variable inflammatory involvement of joints with the subsequent destruction of cartilage and bone. Because of advancements in biomedical technology, new treatments have been developed and have improved patient outcomes considerably in the last 20 years and now include targeted disease-modifying therapies. However, considerable heterogeneity exists between patients in their clinical manifestations, disease course, and response to newer agents. These differences have led some investigators to conclude that rheumatoid arthritis is comprised of a group of disorders with apparent differences in their clinical phenotype and genetic expression that may variably impact their clinical responses to medications [36].

Clinical trials for patients with rheumatoid arthritis also have evolved considerably since the first few US Food and Drug Administration (FDA) approvals of therapeutic agents for RA. The changes that have occurred may be due in part to the increasingly competitive clinical trial landscape, technological advances, and the requirements imposed by regulators over time [41]. Progress in RA clinical trials

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has resulted in more robust clinical trial designs, more appropriate characterization of target subpopulations and more clinically meaningful disease assessments. Nevertheless, evidence-based treatments available for patients with RA have yet to achieve sustainable remissions for the majority of patients [17]. Instead, novel treatments are needed.

Biopharmaceutical companies and others are interested in making go/no go development decisions sooner to enroll trials faster and make effective treatments more available to patients with unmet medical needs. Unfortunately, unless there is a shift in our current approach to research, greater numbers of RA patients will be needed to properly conduct all of the clinical trials currently being planned or underway. Given the complexities of RA diagnosis, assessment, and treatment, the need for sensitive and specific biomarkers is critical. Biomarkers that can help effectively diagnose disease are important as many patients are only diagnosed once permanent damage has started and the time for optimal treatment may have passed [23]. Biomarkers may be necessary to further advance drug development for RA to achieve sustained remissions in disease activity. Biomarkers identify more homogeneous RA populations and allow insight to be gained into individual patient responses. Current biomarkers in RA are diverse and include acute phase reactants, autoantibodies, cell subsets, synovial immunohistochemistry, genetic markers, gene expression markers, cytokines, and growth factors that might be used for diagnosis, prognosis, treatment response, determination of remission, and induction of tolerance. Herein, we will explore some of the biomarkers that have been identified for RA and their current use in clinical trials and discuss important considerations for advancing biomarker detection and utilization in the near future.

2 What Are Biomarkers?

A 2001 joint publication of the FDA and the National Institutes of Health (NIH) has clearly defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention” [5]. This canonical definition contains two critical components; the first is the biological parameter to be measured, and the second is the application of that measurement to a clinical decision or outcome. What is also implied here is that there is a sound method to measure the biomarker. An effective biomarker must be validated for both the robustness of the assay and the utility of the marker. Biomarker assays typically are developed in the lab and tested for assay robustness first in lab models followed by testing in relevant human populations. Testing the utility of the assay may have to be conducted in multiple clinical settings to ensure it will answer the question(s) posed.

Biomarkers can be used in a variety of ways. They may be used to confirm diagnosis of disease and disease stage (remission to severe RA) and to provide a prediction of response to therapy and disease prognosis. These types of markers can be used to stratify patients going into a clinical trial. Response biomarkers are used to monitor the treatment effect of either an approved drug or experimental treatment. Response biomarkers also can be used during clinical trials to help understand drug

Table 1 The definition and characteristics of stratification and response biomarkers

Biomarkers in clinical trials and clinical practice	
Stratification biomarkers	Response biomarkers
Clinical trials: measured before entry into a clinical trial and used to include or exclude patients and/or balance treatment arms	Clinical trials: typically measured at time zero and one or more times during the clinical treatment. Changes are compared to baseline
Patient care: used for patient diagnosis and initial treatment decisions	Patient care: used to monitor response to treatment and adjust treatment
Diagnostic—accurately diagnose disease and disease subclass	Pharmacodynamic (PD)—dynamically assess physiological/biochemical effect of treatment; includes understanding mechanism of action (MOA) and target engagement
Prognostic—predict natural course of disease	Theragnostic—monitor progression and/or response to therapy
Predictive—predict likely response to treatment(s)	Surrogate endpoint—substitute for a clinical efficacy endpoint

mechanism of action (MOA) or as a surrogate endpoint (Table 1). In some cases the same biomarkers are used as both stratification and response markers. Developing new biomarkers and taking them from the bench through clinical trials and into clinical practice can be long and challenging. However, the rewards for RA patients may be quite significant in that the clinician's treatment selection is likely to be more precise and overall patient outcomes better.

3 Biomarkers Currently Used in Rheumatoid Arthritis Clinical Trials

Precision medicine is an emerging approach for disease prevention and treatment that focuses on tailoring prognostic and therapeutic strategies to a patient's individual characteristics [9]. Precision medicine hopes to provide "the right dose of the right drug for the right indication for the right patient at the right time" [12] and is based on a full understanding of the patient's disease and the mechanism of different therapies, as well as empirical evidence linking the two to provide effective treatment guidelines. Due to the large degree of heterogeneity in RA, applying precision medicine will be challenging but potentially very rewarding. The disease heterogeneity in RA is a current limitation to the successful conduct of clinical trials because of the need for increased patient numbers to demonstrate benefit and as such can hinder the discovery of effective evidence-based treatments for use in clinical practice.

Many patients seen by rheumatologists, such as older patients and those with multiple comorbid conditions, are often excluded from clinical trials [6]. While broader inclusion criteria (IC) might help to ensure results are applicable to a larger percent of patients, thereby increasing patient heterogeneity in trials, they also may be more likely to produce inconclusive results [20] and are contrary to the endeavor of precision medicine.

3.1 Biomarkers as Inclusion Criteria for Clinical Trials

The use of biomarkers may allow the identification of more homogeneous subpopulations for enrollment into clinical trials. Understanding the basis of disease heterogeneity and stratifying patients based on effective biomarkers allows trials to enroll an enriched patient population and move toward more precise medicinal treatment. This should lead to increased treatment success rates by allowing trials to meet their endpoints with smaller populations, lower costs, and faster timelines [1, 3, 4]. A biological understanding of RA disease heterogeneity will help both the development of new targeted therapies and finding the correct patient subpopulation for the treatment [25] since homogeneous subpopulations in rheumatoid arthritis may be more responsive to particular therapies that target specific factors playing a role in the pathogenesis of disease. As such, having biomarkers that enable the identification and stratification of distinct RA subpopulations that are related by their underlying disease pathogenesis would likely result in clinical trials that are better designed to answer research questions posed and ultimately allow greater discrimination between treatment cohorts. Doing so will also increase the likelihood of identifying drugs that can induce a sustained remission of disease activity.

Classification criteria for the diagnosis of RA have included biomarkers for many years. The ACR/EULAR rheumatoid arthritis classification criteria include four different biomarkers for use as diagnostic criteria: rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and anti-cyclic citrullinated peptide (anti-CCP). As only a subset of RA patients express many of these biomarkers, their use as inclusion criteria risks the exclusion of some RA patients from trial participation due to the lack of a sufficiently sensitive biomarker profile, despite fulfilling the current guidelines for diagnosis of RA via other criteria, which may be both frustrating to investigators and exert a negative impact on recruitment.

A search of Citeline Trialtrove resulted in identification of 359 Phase I to Phase III trials enrolling RA patients that concluded or will conclude between 1 May 2012 and 2030 (3 years of data for ongoing and planned trials) for which details on the inclusion criteria (IC) were available. Of these, 151 (42.1 %) include at least one mandatory inclusion criterion related to biomarkers (see Fig. 1 and Table 2). The use of biomarkers to define the target patient population varies with study phase but is most frequent in Phase I/II and Phase II studies. Acute phase reactants (ESR and CRP) are the most common biomarkers used as inclusion criteria. Among studies using biomarkers, 135 of 148 studies specified a minimum value for at least one of the acute phase reactants. Although most studies provide acceptable ESR or CRP levels for eligibility, some base eligibility on CRP alone. Due to limitations inherent in the use of acute phase reactants as biomarkers, determination of eligibility based on the ESR or the CRP rather than to one or the other may increase the size of the available RA patient pool [46].

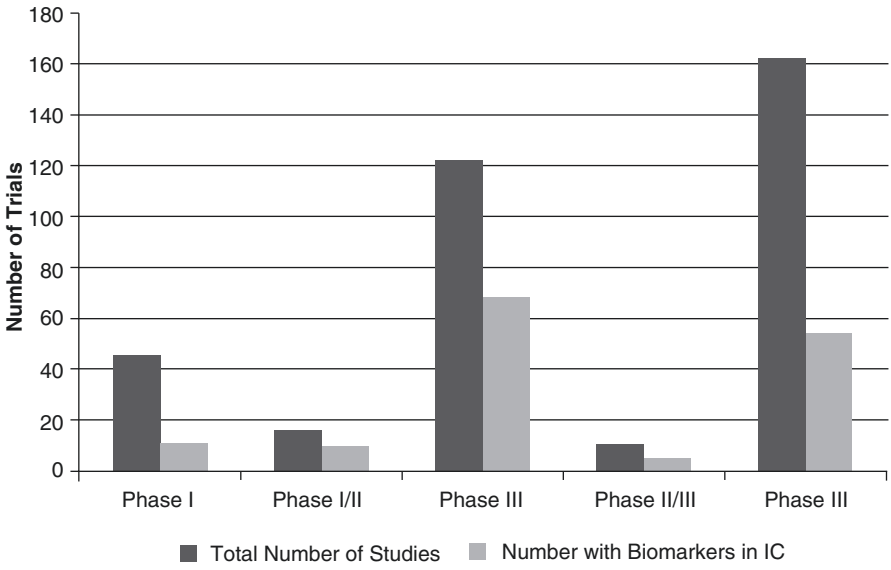

Fig. 1 135 of 359 studies of rheumatoid arthritis included a minimum value of the CRP or ESR as part of the inclusion criteria. *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *IC* inclusion criteria. Use of CRP or ESR in clinical trials for rheumatoid arthritis

Table 2 Summary of biomarkers in recent clinical trials in rheumatoid arthritis

Category	Number of trials	% of total trials
Total number of RA studies	359	–
Total with biomarkers in inclusion criteria	148	41
Total with acute phase reactants[a]	135	38
CRP or ESR	71	20
CRP only	47	13
ESR only	5	1
ESR or CRP or a non-biomarker measure	7	2
Total with anti-CCP and/or RF only	9	3
Anti-CCP[b] or RF	7	2
Anti-CCP only	2	<1
Total with anti-CCP and/or RF in combination with CRP and ESR	50	14

CRP C-reactive protein, *ESR* erythrocyte sedimentation rate, *anti-CCP* anti-cyclic citrullinated peptide, *RF* rheumatoid factor

[a]Five additional studies had more complex inclusion criteria that did not fit into these categories

[b]Two studies required ACPA positivity

4 Use of CRP or ESR in Clinical Trials for Rheumatoid Arthritis

Evaluation of the inclusion criteria for these studies demonstrated the biomarkers used in study inclusion criteria which were primarily limited to ESR, CRP, RF, and anti-CCP. The frequency of individual biomarkers as a criterion in the dataset is presented in Table 2.

The most common biomarker used to determine patient eligibility was CRP, and the most common minimum required CRP value for eligibility was 1.0 mg/dL, although the range of acceptable CRP levels was wide (0.3–2.0 mg/dL). Approximately 20 % of studies defined acceptable CRP levels based on the upper limit of normal (ULN), rather than as an absolute value. Studies defining acceptable CRP levels in relation to ULN most commonly allowed subjects with a CRP >ULN or > 1.2× ULN. Figure 2 presents the frequency of each minimum CRP value for study eligibility and the upper limit of normal CRP is in Table 3.

Selection of an appropriate biomarker inclusion criterion is a challenge in RA. Inclusion criteria requiring comparatively high minimum CRP values are likely to result in increased screen failure rates, which may delay achievement of recruitment goals and cause frustration for investigators and potential participants. Low minimum values risk inclusion of patients with only low basal disease activity or who may have disease that cannot improve. On the other hand, since the presence of anti-citrullinated protein antibody (ACPA) and their concentration at baseline has been shown to be strongly predictive of radiographic progression, higher values as an inclusion criterion are important in the evaluation radiographic outcomes [18, 37].

Requirements for RF or anti-CCP antibody positivity were present in the inclusion criteria for fewer studies than CRP or ESR; only 59 studies required RF or anti-CCP antibody positivity, primarily in the Phase II and Phase III settings. Interestingly, studies requiring antibody positivity frequently also specified inclusion criteria related to ESR or CRP values (85 %).

5 Diagnostic Biomarkers

Multiple biomarkers have been shown to be useful in confirming the diagnosis of RA, both in clinical practice and in the clinical research setting. Recognition of the value of biomarkers in the diagnostic process is exemplified by the inclusion of both serology (RF and/or anti-CCP) and acute phase reactants (CRP and/or ESR) in the ACR/EULAR 2010 rheumatoid arthritis classification criteria [2]. A diagnosis of definite RA requires evaluation of at least one serological test and one acute phase reactant. Rheumatoid factor and anti-CCP, however, may perform better as diagnostic tests if they had greater sensitivity and specificity. Consequently, there remains a need for additional diagnostic biomarkers with greater sensitivity and specificity than those that have been used to date, as well as biomarkers that allow the identification

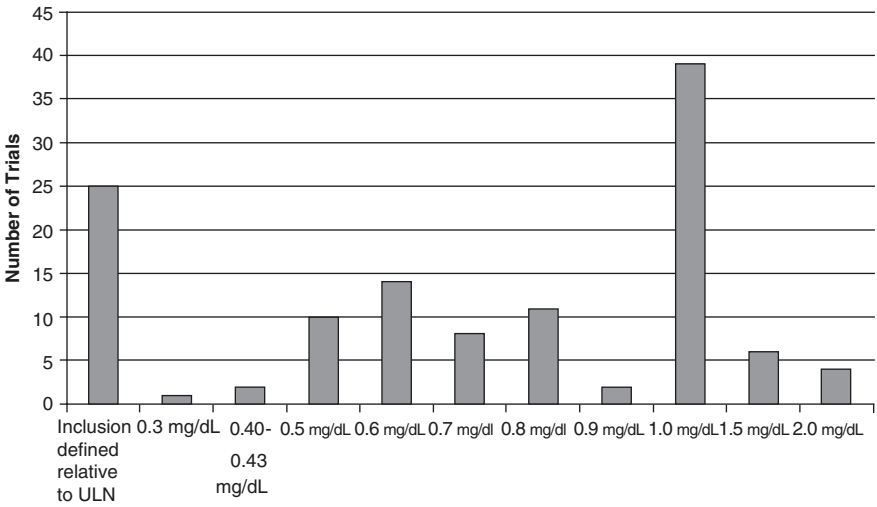

Fig. 2 Minimum CRP value for inclusion. Minimum CRP values required per trial inclusion criteria

Table 3 The C-reactive protein (CRP) upper limit of normal was specified in 25 of the clinical trials inclusion criteria based on the upper limit of normal (ULN)

Required CRP for eligibility relative to upper limit of normal (ULN)	Number of trials
> ULN	11
>1.2× ULN	11
>1.4× ULN	1
>1.5× ULN	2

of more homogeneous subpopulations of patients with rheumatoid arthritis to determine the benefit of therapies to which they are more likely to respond. Novel biomarkers or combinations of biomarkers with better operating characteristics that have been identified may allow research subject stratification within clinical trials, the diagnosis of patients with early disease, and the identification of patients in clinical practice that are more likely to achieve the goals of treatment. However, additional testing and validation in RA are needed.

6 Rheumatoid Factor

Rheumatoid factor, an autoantibody targeting the Fc region of IgG, is among the most widely used biomarkers in RA diagnosis. Although it is widely used and a valuable tool in diagnosis of RA, there are limitations to the use of RF as a diagnostic test.

Table 4 Sensitivity and specificity of RF and/or anti-CCP antibody for the diagnosis of RA

Biomarker	Sensitivity (%)	Specificity (%)
RF	91.7	74.4
Anti-CCP antibody	88.0	90.4
RF + anti-CCP antibody	90.2	83.3

RF rheumatoid factor, *anti-CCP* anti-citrullinated protein antibody

Notably, RF is not specific to RA and is elevated in other immune-inflammatory diseases as well as in certain infections. A meta-analysis that included IgM, IgA, and IgG RF isotypes to assess the diagnostic accuracy of anti-CCP and RF for RA demonstrated qualitative similarity between them [31]. The pooled sensitivity and specificity of IgM RF for RA were 69 % and 85 %, respectively. A small study [19] demonstrated that 70 % of RA patients positive for RF had elevation of two or more isotypes, compared to 16 % of RF positive patients with other rheumatic conditions, and that IgM and IgA RF antibodies in combination were significantly more common in RA patients than in patients with other rheumatic conditions, suggesting that determining the presence of multiple isotypes of RF antibodies may provide increased specificity for RA.

A recent prospective cohort study in Denmark demonstrated that individuals with elevated plasma IgM RF levels are at increased long-term risk of developing RA, and this risk increased with increasing RF levels—a finding that could be beneficial in identifying patients prior to the onset of clinically significant disease [30]. Notably, IgA RF may be present years before the onset of clinical symptoms, although specificity is comparatively low (Rantapää-Dahlqvist 2003).

In 2015, the value of RF with and without coexistent ACPA was assessed for the diagnosis of RA [35]. They evaluated 135 subjects with RA who were outpatients or inpatients over a 1 year period and compared their results to 50 healthy patients who underwent physical examinations in their hospital during the same period. The sensitivity and specificity of RF for the diagnosis of RA were 91.7 % and 74.4 %, respectively, while that for anti-CCP antibody were 88.0 % and 90.4 %, respectively. For the combined detection of RF and anti-CCP antibody, the sensitivity and specificity were 90.2 % and 83.3 %, respectively (Table 4).

7 Anti-citrullinated Protein Antibodies

Anti-citrullinated protein antibodies (ACPAs) are highly specific to RA, a distinct advantage over RF. Anti-CCP is the most common ACPA in the current use and may be present years before the onset of clinical symptoms and may increase in frequency closer to disease onset. Anti-CCP antibodies are present in a greater percentage of RA patients than RF in most settings, the exception being early RA (IgM RF present in 73 % vs. 70 %). Detection of both anti-CCP and RF antibodies prior to symptom

onset has resulted in specificities approaching 100 % [34]. On the other hand, sensitivities of these combinations prior to onset of symptoms remain low (range: 6–52 %), a known limitation of anti-CCP in the diagnostic setting (Rantapää-Dahlqvist 2003). Anti-CCP antibodies have been shown to have the greatest diagnostic performance and have been recommended for consideration as a first-line screening technique [31].

The presence of ACPAs has been associated with a more aggressive disease course than is observed in ACPA negative disease. A study of 454 patients with early RA in the Netherlands demonstrated that although patients had similar symptoms at inclusion, anti-CCP positive patients had significantly higher radiological scores, as well as a larger number of swollen joints after 4 years of follow-up, although the distribution of erosion scores, joint space narrowing, and inflamed joints in the hands was similar between the groups [44].

Although the presence of anti-CCP and RF typically is associated with aggressive disease, recent clinical evidence suggests that this outcome can be modulated in patients with early RA. Data from a randomized, placebo-controlled clinical study in Sweden demonstrated that in patients randomly assigned to receive low-dose prednisolone (7.5 mg/day) or placebo for 2 years, the presence of RF and anti-CCP antibody predicts radiographic progression in only the placebo group [14]. Similar findings were reported from an analysis of data from the Combination Anti-rheumatic Drugs in Early RA (CARDERA) trial, in which 467 patients with early, active disease were assigned to receive methotrexate, methotrexate + cyclosporine, methotrexate + prednisolone, or methotrexate + cyclosporine + prednisolone. Among subjects positive for ACPA, treatment with any of the study treatment options resulted in a statistically smaller change in Larsen score relative to ACPA negative subjects. In contrast, no statistically significant change in Larsen scores for any treatment arm was observed in the ACPA negative group relative to placebo, and, overall, the change in Larsen scores over the 2-year study period was smaller in the ACPA negative group compared to that observed for ACPA positive patients (Seegobin 2014). These studies provide evidence of the potential for diagnostic biomarkers to impact the disease state in patients with RA. In addition to anti-CCP, other citrullinated proteins that may be targeted by antibodies include perinuclear factor, keratin, vimentin, fibrinogen, histones, MBP, type II collagen, and α-enolase. Anti-carbamylated protein antibodies (anti-CarP), including those recognizing homocitrulline, carbamylated fibrinogen, or carbamylated vimentin, also serve as biomarkers for RA, although the sensitivity of these antibodies is lower than that of the ACPAs [10, 15, 29]. Approximately 43 % of patients with RA are positive for IgG anti-CarP antibodies and 45 % for IgA anti-CarP antibodies [39, 40]. Also of note, the presence of anti-CarP antibodies was noted in some patients who were negative for ACPA antibodies and appears to correlate with a more severe disease course [40]. Anti-CarP antibodies may be detectable prior to the diagnosis of RA [40] and, therefore, may have potential utility in identifying patients with early disease.

Table 5 Corresponding disease activity score (DAS)28-erythrocyte sedimentation rate, DAS28-C-reactive protein, sensitivity, and specificity values derived from the receiver operating characteristic curves for each criterion

Criteria	DAS28-ESR	DAS28-CRP	Sensitivity	Specificity
Remission	2.6	2.32	0.921	0.869
Low disease activity	3.2	2.67	0.908	0.893
High disease activity	5.1	4.09	0.925	0.970

Ann Rheum Dis 2007; 66:407–409
CRP C-reactive protein, *DAS* disease activity score, *ESR* erythrocyte sedimentation rate

8 14-3-3

The proteins 14-3-3 eta and gamma have been demonstrated to be elevated in synovial fluid and serum of patients with inflammatory joint disease relative to control subjects [21, 26]. Recent work by Maksymowych et al. [27] suggested a role for 14-3-3η as a potential diagnostic biomarker for rheumatoid arthritis. The authors demonstrated sensitivity of 63 % and specificity of 93 % for 14-3-3η alone as a diagnostic marker for early RA versus healthy controls and sensitivity of 77 % and specificity of 93 % in established RA via an ELISA-based assay. The combination of 14-3-3η, ACPA, and RF was found to have specificity of 78 % in early RA versus 71 % for ACPA and RF alone. However, the sensitivity of 14-3-3η, ACPA, and/or RF was 78 %, as compared to 84 % for RF and/or ACPA alone.

8.1 Disease Activity Biomarkers in RA

Measures of disease activity including the DAS28 (ESR, CRP) and the SDAI use a combination of tender and number of swollen joints and global assessments of disease activity and include the ESR or CRP to produce an overall disease activity score (Table 5).

In addition to the use of biomarkers for confirmation of diagnosis and assessment of disease activity, these and others have recently been used to predict the response to therapy. Understanding the operating characteristics of existing biomarkers and those being studied will enable their application and utilization in the most effective manner possible.

9 Vectra® DA

Recent investigators have evaluated panels of proteins in the assessment of rheumatoid arthritis disease activity. The Vectra® DA blood test integrates the concentrations of 12 distinct protein biomarkers consisting of vascular cell adhesion molecule-1, epidermal growth factor, interleukin-6, tumor necrosis factor receptor

type I, matrix metalloproteinase 1, matrix metalloproteinase 3, human cartilage glycoprotein-39, leptin, resistin, serum amyloid A, and CRP into a single score between 1 and 100 that indicates the current level of RA disease activity based on an algorithm [38]. The numerical score is reported along with a classification of the disease into low (<30), moderate (30–44), and high (>44) disease activity. Currently, the Vectra® DA score in Phase II and III clinical trials is increasingly being utilized as an independent inclusion criterion for disease activity and is being evaluated for response to novel disease-modifying antirheumatic drug (DMARD) therapy as a secondary or exploratory variable.

A recent study evaluating RA subjects with and without fibromyalgia demonstrated similar levels of disease activity between the CRP and a multi-biomarker disease activity score using the same reagents and algorithm as the Vectra® DA score (MBDA), whereas the patient global assessment and the DAS28-CRP were significantly greater [24], suggesting the possibility that it may be a better disease activity measure than some of the parameters currently being used in clinical trials. These findings, however, are not particularly surprising and are consistent with findings in previous studies in which radiographic progression was assessed in relation to DAS28-CRP and MBDA scores [45]. Among subjects who achieved a DAS28-CRP remission, those continuing to have a high MBDA score (>44) were more likely to have joint progression during the subsequent year as opposed to those with an MBDA score in the remission range (≤25). Similarly, another study evaluated the ability of an MBDA score using the same algorithm as the Vectra® DA score at baseline to predict progression in radiographic joint damage in DMARD-naïve early RA subjects in whom a treat to target strategy was being used [28]. The latter study further demonstrated that the MBDA score independently predicted progression in radiographic joint damage and that subjects with higher MBDA scores were more likely to have progression in radiographic joint damage.

10 Validation of Rheumatoid Arthritis Biomarkers

The Outcome Measures in Rheumatology (OMERACT) initiative has worked on validating tools for evaluating the effect of therapeutic interventions in rheumatic diseases since 1992. The OMERACT initiative identified important questions to address with respect to imaging and soluble biomarkers [11]: first, whether the outcome measure relates to the suspected pathophysiological change; second, whether the measure has an agreed and consistent procedure; and third, to what extent operator expertise is a prerequisite. Importantly, it was recognized that while the CRP has been demonstrated to be sensitive to change and to fulfill most of the aspects of truth for therapeutic purposes, insufficient data existed for other proposed soluble biomarkers, and further validation was needed for recommendations to be made.

Recent draft guidance from FDA states that, “Biomarkers can be used for a wide variety of purposes during drug development; therefore, a fit-for-purpose approach should be used when evaluating the extent of method validation that is appropriate.

When biomarker data will be used to support a regulatory action, such as the pivotal determination of safety and/or effectiveness or to support labeled dosing instructions, the assay should be fully validated" [43]. Requirements for validation involve measuring an assay with well-established performance characteristics and agreement on the physiologic, toxicologic, pharmacologic, or clinical significance of the results [13]. Once in the clinic, both analytical validation of the assay (accuracy of the measurement versus a gold standard) in patients and clinical validation (correlation with the clinical endpoint) must be completed.

11 Technological Advancements in Testing for Biomarkers

Several technological and scientific advancements are aiding in both the discovery and development of new therapies and biomarkers. These include sequencing of the human genome and access to next-generation sequencing (NGS), improved technologies for biomedical analysis, and new tools for using large datasets [8, 9]. These trends are affecting all disease areas, including biomarkers for RA.

Sequencing the human genome and NGS has revolutionized the field of genetics and genomics and provides virtually limitless data to investigate the genetic causes of diseases. As these technologies mature, rapidly decreasing costs further enhance their value to drug development. The cost of sequencing a single whole-human exome has dropped well below $5,000, and it continues to fall, although analysis and informatics costs are not figured into that number. Several trends have made the data more available as well, such as an increasing informed and proactive consumer and NGS being directly marketed to consumers. Based on NGS, several disease-associated genes have been linked for rheumatic diseases in both case-control and family-based studies [47], although much work will need to be done to explore whether they are causative variants. Future scientific advancements, including multiple technology platforms and multifactorial testing (multi-gene or multi-analyte signatures), will increase our ability to interrogate the molecular pathways involved in common and complex diseases.

12 Main Challenges in Biomarker Discovery

Due to the progressive nature of RA, an early diagnosis, prognosis, and treatment of the disease are needed, especially in patients without clear manifestation. Early-stage diagnostic and prognostic biomarkers will facilitate clinical practice decisions and selection of the appropriate populations in clinical trials. Strategies to improve the predictive value of biomarkers include combinations of biomarkers and the use of imaging techniques in combination with biomarkers.

Usefulness of biomarkers depends on biomarker discovery, their availability in practice, and their validation at the time of their need. While the use of biomarkers

to understand disease pathophysiology and for diagnostic and prognostic purposes is more direct, strict qualification and clinical validation are a must in order to support approval of medicines. Furthermore, few biomarkers are accepted as a surrogate endpoint. The validation of biomarkers in RA and their cut points is a major challenge and will need coordinated efforts from the regulatory authorities, academia, and industry consortia. The same collaborative approach is needed to demonstrate the translation of the use of a biomarker into actual clinical benefit for the patients. The relationship of biomarkers with relevant clinical outcomes requires large sample sizes and very meticulous observation. Clinical outcomes, patient-reported outcomes, and disability also should be considered to assess the value of a biomarker or a treatment strategy that employs biomarkers in decision-making. New technologies and statistical methodologies are facilitating the discovery of biomarkers at a much faster pace. Their rapid assessment to determine their operating characteristics will be important to advance clinical research. On the other hand, complexity (biomarker panels) may be a barrier to the application of biomarkers, especially if more wide-scale profiling aims to guide medical decision-making. The high costs of testing and limitations of access to new technologies will require the demonstration of significant cost-benefit before they are broadly accepted by multiple stakeholders.

13 Emerging Trends in Biomarkers

Well-organized and agile consortia from academia and industry will be essential to identify and validate new biomarkers. The rapid progress in the fields of biotechnology, genetics, and genomics and their integration in clinical practice and in product development require collaboration from a variety of different stakeholders and disciplines.

Biomarkers will be fundamental tools not only to demonstrate proof of concept but also for establishing the required dose and dose window and improving the effectiveness of classical dose-finding studies based on clinical efficacy and safety. A deep understanding of the molecular basis of disease and dynamics of response to treatment is needed to assess the relationship between pharmacodynamic (PD) effect and downstream clinical effect.

Innovative approaches to increase efficiency in clinical trials are currently being used [42]. Adaptive clinical trial designs aim to introduce flexibility and facilitate decision-making during the implementation of a trial. Practical examples that have been used in other disease indications (e.g., oncology) can be applied to rheumatoid arthritis. An umbrella protocol is designed to allow enrollment of patients into different treatment arms based on their specific biomarker profile within the same indication [22]. Randomization to different drugs, combinations, or dosing strategies can be stratified according to the subjects' biomarker profiles. Biomarkers are the essential instruments that allow a personalized medicine approach to the application of patient-specific profiles based in biomarkers and clinical factors to assess

individual risks and prognosis and to provide tailored prevention and disease-management strategies.

Understanding molecular medicine based on a single biomarker does not address the full picture of the connections and feedback between closely related pathways. As a consequence there is a need to integrate combinations of biomarkers from related pathways to increase the predictive value. The Vectra DA is an example, combining measurements of 12 serum proteins to calculate a multi-biomarker disease activity score. In addition, the integration of different technologies, for example, imaging techniques in combination with biomarkers, may improve early diagnosis of RA particularly in seronegative patients and the assessment of response to therapy.

The application of "big data" to biomarkers in rheumatoid arthritis may yield benefit at three levels: descriptive models to gain information and knowledge, predictive models to better understand what will happen in the future, and prescriptive models to provide recommendations for decision-making. Trial simulations, virtual trials, and strategy trials are additional examples of the potential utility of big data. The inclusion of different biomarkers in the database should facilitate estimation of their usefulness and potential.

Personalized medicine with a biomarker foundation will produce changes in the reimbursement policies. In a heterogeneous disease such as rheumatoid arthritis where numerous expensive drugs are available, personalized medicine would have an impact on drug budgets. Linking research and electronic health records can strategically optimize patient segmentation, clinical development, and health outcomes. Moreover, patient stratification in the real world may enable a medication to increase effectiveness and achieve the reimbursement that would not be achieved in a broader population. Consequently, a new dimension is now highly relevant for biomarkers: how the biomarkers behave across a large number of patients and their effectiveness in real-world personalized medicine.

14 Potential Investment Required for Use in Clinical Trials

Currently, biomarkers fall short of what is needed to change our approach to clinical trials for rheumatoid arthritis. The use of a combination of biomarkers, new technologies, and multidisciplinary approach requires heavy investment. DNA sequence data alone is not enough in complex diseases as rheumatoid arthritis and different data are now of interest beyond DNA sequence: DNA methylations, SNPs, protein-coding RNA, noncoding RNA, histone modifications, transcription factors and their DNA binding sites, transcription start sites, promoters, protein-protein interactions, protein modifications, and metabolites. Investment in these technologies is only the first step since the data they generate require the use of a systems biology approach to data integration.

Additional requirements include investments in tools and resources that allow merging of data from biomarker research with data from health care and clinical

trials as well as investments in informatics systems that enable the analysis of diseases and therapeutic interventions. One example of this is Project Data Sphere (www.projectdatasphere.org) [33], a database that allows researchers affiliated with life science companies, hospitals, and institutions, as well as independent researchers, to share, integrate, and analyze patient-level, comparator arm, Phase III cancer de-identified data.

15 Ethical and Legal Considerations

Respect for human dignity of all individuals voluntarily participating in human research and donating biological materials is mandatory and correct. The four conventional bioethical principles of autonomy, beneficence, non-maleficence, and justice should be ensured. In that respect, research based in biomarkers without careful consideration of the ethical principles may affect those principles when they have an effect on patient selection, access to clinical trials, access to medications, and data privacy. The use of biomarkers has an effect beyond the utility in product development. In the near future, new technologies and cost reductions will make available the whole-genome sequencing as a standard test. The consequences of the generalized use of biomarkers and genetic tests are clear. False positives or false negatives may have an impact in people's lives when it affects prognosis, access to treatments, stigmatization, insurance reimbursement, and work opportunities.

Genetic testing is heavily regulated, but research using nongenetic biomarkers should follow strict procedures as well. Local and international deontological codes, research guidelines, data protection laws, and regulatory directives should be followed in biomedical research. The use of stored biological materials of human origin is a powerful tool in biomarker research. The benefit of this secondary research goes beyond the individual and may improve human health and health-care systems. If stored samples were not used, the alternative prospective is the collection of new biological materials specifically for each project. Nevertheless, this effort would not be feasible in many cases or would be too costly and would take a long time, making unavailable the benefits of research to the health system or delaying those benefits for years. New knowledge brings new hypothesis and induces new uses and analyses of stored biological materials. Despite the controversies regarding the limitations for research, existing regulations regarding the use of stored material in full respect for private life should be considered. In order to find a balance, there are some guidelines in which the use of stored samples may be approved legally and ethically [7]. In cases where the consent for a further use of stored samples is lacking, reasonable efforts to contact the subject to obtain specific consent to use materials and personal data should be taken. If the person concerned cannot be contacted and there is no known objection from the subject, the use of the samples and data may be granted upon independent confirmation that the following conditions are met: the research is of important scientific interest and the expected scientific benefits support the proportionality principle between the rights of the person concerned and those expected

benefits; the objectives of the research cannot reasonably be achieved using other prospectively obtained biological material [7].

Anonymized, non-identifiable biological materials and data also may be used for secondary research use unless such use was not limited by the subject providing the materials and data and does not violate any law or restrictions placed by the person concerned. The objectives and methods of secondary research with non-identifiable data should be ethically evaluated as well.

Big data brings new legal and ethical issues that affect individuals and communities in different ways. Big data generates secondary uses of data from disparate sources, including research, clinical, registry, administrative, claims, and patient-generated data. Protected health information is an individually identifiable information relating to an individual's care or past, present, or future physical or mental health condition or payment for care. Individually identifiable information directly identifies a person or contains information that permits identification, and big data may increase the possibilities to identify individuals. Legal security and breach notification rules apply differently for regulated entities and public administration than for private users. Security measures should be applied to reasonably and appropriately protect electronic records at the administrative, physical, technical, and organizational levels. Information may be de-identified by different methods, including the removal of 18 specific identifiers ("Safe Harbor" method; [32]), or by expert determination that there is minimal risk that information could identify individuals ("Statistical" method; [16]). Nevertheless, de-identified data is not useful for all research and some biomarkers, as genetic information, are considered identifiable information. Disclosure and the use of identifiable patient data is allowed if there is patient consent. But there is a lack of a consistent framework for patient consent, and requirements vary depending on the type of information and intended use. New ways to get patient approvals are needed, and there is a major shift in public perceptions of privacy as social use of the Internet is spreading. Therefore, ethical and legal considerations are expected to change in the future and affect the way research based in biomarkers and share of data will be performed in complex diseases such as rheumatoid arthritis.

16 Conclusion

Rheumatoid arthritis is a heterogeneous, systemic, autoimmune disease that will likely require the identification of more homogeneous subpopulations to achieve the desired treatment goals. Biomarkers in RA may allow earlier diagnosis, the prediction of responses to therapies, and advancements in clinical trial design. Biomarkers should be an essential part of a precision medicine approach that focuses on tailoring prognostic and therapeutic strategies to a patient's unique underlying disease profile. Traditional RA and novel biomarkers offer the potential to advance care especially when combined with robust data linking biomarker signatures to successful outcomes.

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