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Biomarkers of Rheumatoid Arthritis Activity: From Traditional Indicators to Molecular Predictors of Therapeutic Response

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Abstract: This review presents a systematic analysis of biomarkers of rheumatoid arthritis (RA) activity, focusing on their prognostic and clinical relevance. The work characterizes classical inflammatory indicators (CRP, ESR), immunological markers (rheumatoid factor, anti-CCP, anti-MCV), cytokine profiles (IL-6, TNF-α, IL-17, IFN-γ), as well as molecular predictors including anti-CarP, carbamylated proteins, microRNAs (miR-146a, miR-155), genetic alleles (HLA-DRB1*04:01, PTPN22), and proteomic panels. Clinical data on over 15 key multi-omics predictors of therapeutic efficacy with methotrexate, tocilizumab, JAK inhibitors, and leflunomide are analyzed. Special attention is paid to the sensitivity and specificity of biomarkers in the assessment of DAS28, CDAI, and radiographic progression according to the Sharp/van der Heijde method. A direct association was found between IL-6 levels >30 pg/mL and active disease phase and erosion risk, as well as the role of anti-CCP2 titers >60 U/mL in predicting a destructive phenotype. A multidisciplinary synthesis of data from over 100 domestic and international sources published between 2007 and 2025, including meta-analyses, population-based cohort studies, and EULAR/ACR recommendations, is presented. The review critically evaluates stratification approaches based on biomarker platforms and their potential integration into clinical algorithms for personalized RA therapy.

Keywords: rheumatoid arthritis, IL-6, TNF-α, anti-CCP, microRNA, anti-CarP, HLA-DRB1, molecular predictors, JAK inhibitors, DAS28, therapeutic biomarkers, immunodiagnostics, clinical stratification.

Introduction

Rheumatoid arthritis is one of the most extensively studied systemic inflammatory diseases, yet it remains challenging in terms of controlling disease activity and predicting clinical response to therapy. Classical biomarkers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and anti-CCP antibodies, are commonly used to assess inflammation. However, their prognostic value varies depending on the disease stage and associated immune phenotypes. CRP levels above 10 mg/L and ESR above 40 mm/h are indicative of high disease activity, but they do not reliably predict the effectiveness of genetically engineered biological agents (GEBAs) or Janus kinase inhibitors.

Immunological indicators, including anti-MCV, anti-CarP, and anti-carbamylated antibodies, have shown a stronger association with the destructive course of RA. Elevated anti-CCP2 titers above 60 IU/mL are associated with erosive damage to the small joints of the hands within the first 12 months. The production of proinflammatory cytokines—particularly IL-6 and TNF-α—exceeding thresholds of 30 pg/mL and 25 pg/mL respectively, demonstrates a significant correlation with DAS28 and CDAI indices, as well as with the degree of synovial hypertrophy as observed on ultrasound and MRI.

In recent years, research efforts have intensified to identify microRNAs (miRNAs) as biomarkers of disease activity and therapeutic response. Expression levels of miR-146a and miR-155 in synovial fluid and serum of patients with active RA are more than five times higher than in the control group, reflecting the involvement of Toll-like receptors and the NF-κB cascade in chronic inflammation. Additionally, the expression of genes such as HLA-DRB1*04:01, PTPN22, and STAT4 contributes to persistent immunopathological responses and is predictive of methotrexate treatment failure in patients with disease duration longer than six months.

Given the high inter-individual variability in clinical response, there is a growing need to move from a unified disease activity assessment model to a stratified approach, integrating molecular and cellular biomarkers. This review article systematizes data on both traditional and novel biomarkers of RA activity, emphasizing their quantitative characteristics, sensitivity, specificity, and utility in predicting response to specific therapeutic agents. The analysis encompasses clinical and experimental data derived from over 40 cohort studies and 25 multicenter randomized trials published between 2007 and 2025.

Materials and Methods

This review is based on the analysis of 113 sources, including 45 original clinical studies, 28 systematic reviews and meta-analyses, 25 randomized controlled trials, and 15 clinical guidelines and consensus documents published between 2007 and 2025. Only studies presenting quantitative data on RA activity biomarkers and their correlation with validated disease assessment tools were included. These tools include DAS28, CDAI, SDAI, Sharp/van der Heijde score, Larsen index, C-reactive protein level (mg/L), ESR (mm/h), serological markers (anti-CCP, anti-MCV, anti-CarP, RF), serum cytokines (IL-6, TNF-α, IL-17, IFN-γ), and expression of miR-146a, miR-155, HLA-DRB1*04:01, PTPN22, and STAT4.

Articles were selected based on the application of standardized biomarker measurement techniques, including multiplex immunoassay (Bio-Plex), immunochemiluminescent assay (IMMULITE), real-time polymerase chain reaction (RT-qPCR), next-generation sequencing (NGS), and proteomics using LC-MS/MS. Studies were included if IL-6 levels were measured with a sensitivity threshold of ≤0.5 pg/mL, TNF-α with ≤1.0 pg/mL, and the expression levels of miR-146a and miR-155 were normalized to U6. Biomarkers were evaluated in relation to therapeutic responses to methotrexate, tocilizumab, leflunomide, etanercept, adalimumab, and baricitinib, with outcome measures including ACR20/50/70, EULAR response, and sustained remission (DAS28 < 2.6) over 12–24 weeks.

Statistical significance of biomarkers was assessed using thresholds of $p < 0.05$, AUROC > 0.75, odds ratio (OR) ≥ 2.5, and sensitivity/specificity of at least 70%. Only studies with a sample size of ≥80 patients, a confirmed RA diagnosis based on ACR/EULAR 2010 criteria, and control groups (healthy donors or patients with osteoarthritis) were included. Publications lacking precise laboratory parameters, demonstrating poor data reproducibility, or missing correlation or regression analysis were excluded.

Methodological quality was evaluated using the Jadad scale for randomized controlled trials, the Newcastle–Ottawa Scale for cohort studies, and AMSTAR-2 for systematic reviews. Secondary data processing was conducted using RevMan 5.4 software, with intergroup comparisons (t-test, χ^2 test), ROC curve construction, and determination of biomarker thresholds with the highest

prognostic value. Based on the metrics obtained, biomarkers were classified into diagnostic, prognostic, and therapeutically significant models of rheumatoid arthritis activity.

Literature Review

An analysis of the literature demonstrates the evolution in the understanding of biomarkers' role in stratifying rheumatoid arthritis (RA) activity — from non-specific inflammatory indicators to molecular predictors that characterize immune response, risk of joint destruction, and likelihood of remission. In the early stages of research, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were the primary reference points, reflecting the degree of systemic inflammation. Patients with DAS28 > 5.1, median CRP levels reached 42 mg/L, whereas in those with moderate activity, the levels were below 15 mg/L. However, in 25–30% of RA patients with active disease, CRP remained within the normal range, thereby limiting its diagnostic and prognostic value [2].

Serological markers such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP) gained widespread clinical use. The sensitivity of anti-CCP in confirming diagnosis is 68–72%, while specificity exceeds 95%. Anti-CCP levels > 60 U/mL have been associated with a higher risk of erosive joint damage according to MRI and radiographic findings. Early detection of high anti-CCP2 titers, especially in combination with RF, is a strong prognostic factor for progressive disease in patients with a disease duration of less than six months [6].

The cytokine profile has emerged as a functional biomarker of inflammation and therapeutic response. Patients with high RA activity, IL-6 levels exceeded 30 pg/mL; after four weeks of tocilizumab therapy, IL-6 levels dropped by more than 65%. IL-6 and TNF-α levels positively correlate with CDAI scores and the number of swollen joints, while IFN-γ is associated with systemic manifestations. IL-6 concentrations > 20 pg/mL are predictive of methotrexate treatment failure and indicate the need for early initiation of anti-IL-6 or JAK inhibitor therapy [1].

Antibodies to modified proteins, including anti-CarP and anti-MCV, have gained clinical and prognostic relevance. The presence of anti-MCV antibodies increases the risk of radiographic progression by 42% during the first 18 months from disease onset. Anti-CarP antibody levels positively correlate with Larsen score values ($r = 0.51$) and the number of erosions assessed by the Sharp method, regardless of serostatus [10].

Epigenetic and post-transcriptional regulators—particularly microRNAs miR-146a and miR-155—are also considered potential predictors of disease activity and response to remission-inducing therapy. miR-146a expression in patients with DAS28 > 5.1 was 3.6 times higher than in those with clinical remission. Patients resistant to biologic DMARDs (bDMARDs) maintained elevated miR-155 levels ranging from 4.2 to 5.8 ng/mL, with a median DAS28 score of 5.6 [3].

Genetic predisposition and immune-related polymorphisms are also well described in the literature. The allele HLA-DRB1*04:01, increases the risk of aggressive RA progression by 3.2 times, especially in smokers and anti-CCP positive individuals. A statistically significant association between PTPN22 expression and an increased frequency of poor response to TNF-α inhibitors ($OR = 2.9$, $p < 0.001$).

Multi-omics models incorporating proteomic, transcriptomic, and metabolomic panels have been increasingly applied in recent clinical trials. A 12-serum protein panel—including S100A8/9, MMP-3, and YKL-40—predicts response to IL-6 inhibitors with an accuracy of 81.3% and an AUROC of 0.83. Stratification of patients into molecular subtypes of RA was proposed, identifying high-risk clusters characterized by hyperexpression of CXCL13, STAT1, and IFI44L genes [14].

A review of clinical guidelines (ACR, EULAR, and RAR) from 2010 to 2023 confirms the growing importance of molecular diagnostics in therapy selection and remission monitoring.

According to Aletaha D. et al. (2010), the inclusion of serological and cytokine markers in diagnostic criteria increased the sensitivity of early RA classification by 14%. The use of biomarkers has also shown benefits in reducing ineffective treatments, accelerating remission achievement, and optimizing cost-effective use of biologic agents.

Further literature analysis confirms that molecular biomarkers not only refine assessments of RA activity but also predict disease progression, including extra-articular manifestations. 36.7% of patients with elevated IL-6 and IL-17 levels exhibit extra-articular vasculitis, serositis, and interstitial lung disease. Notably, IL-6 levels above 32 pg/mL were directly associated with hospitalization risk within the first 18 months of disease [11].

Patients with IFN-γ > 20 pg/mL and anti-CCP > 100 U/mL had a 29.4% incidence of interstitial fibrosing alveolitis, compared to only 9.6% in the control group without pronounced cytokine imbalance [7].

Research focused on inflammatory anemia in RA suggests that IL-6 is a central mediator of hypoferremia. IL-6 levels > 28 pg/mL were associated with decreased serum ferritin and increased hepcidin. Notably, 61% of these patients had anemia refractory to iron supplementation. These findings were corroborated by Ben-Hadj-Mohamed M. et al. (2017), who reported an inverse correlation between IL-6 levels and hemoglobin ($r = –0.59$; $p < 0.01$), even when ferritin was normal and soluble transferrin receptor levels were low [9].

Genetic and epigenetic studies continue to expand the array of potential predictive factors in RA. A significant association between the HLA-DRB1*04:01 allele and a severe erosive form of RA in the Uzbek population. The combination of this allele with anti-CCP titers >75 U/mL increased the risk of severe joint deformities within the first two years by 4.1 times (OR = 4.1; $p < 0.001$). Patients carrying the HLA-DRB1 allele and elevated anti-CarP levels showed advanced joint destruction by the 12th month of treatment, despite standard methotrexate-based therapy [13].

An important component of disease prognosis is the interaction of immune status with environmental triggers. Patients residing in ecologically stressed areas of Uzbekistan, particularly the Fergana Valley, exhibited cytokine profile changes, with predominance of IL-17 and IL-1β. These results are supported by international surveillance from Cai Y. et al. (2023), which showed that populations in air-polluted clusters had elevated IL-6 and TNF-α levels and worse functional outcomes (HAQ > 1.6).

In the context of biomarker-guided therapy, cytokine levels respond variably to targeted interventions. Panasyuk E.Yu. et al. (2011) reported that in patients treated with tocilizumab, IL-6 levels decreased from an average of 38.2 to 12.5 pg/mL by week 4, whereas methotrexate monotherapy achieved a reduction of no more than 18% over the same period. In a study by Emery P. et al. (2008), combined therapy with methotrexate and etanercept led to a sustained decrease in TNF-α from 27 pg/mL to below 8 pg/mL, with 63% of patients achieving an ACR50 response by week 24.

Incorporating biomarker panels (cytokines, anti-CCP, microRNAs) into the initial RA management algorithm enabled more personalized therapy selection and reduced treatment switching during the first 12 months from 47% to 21%. This is corroborated by Guzzo M.P. et al. (2019), where biomolecular stratification achieved sustained remission (DAS28 < 2.6) in 52.8% of patients, compared to 36.4% in the standard care group [15].

Clinical and prognostic studies increasingly reveal a clear association between molecular biomarker profiles and the rate of joint erosion development. In a study involving 146 patients with early confirmed RA, those with anti-CCP titers >80 U/mL and IL-6 >35 pg/mL showed a 22.7% increase in joint destruction on radiographs (Sharp score) over six months compared to patients with normal values. In contrast, ESR and RF were not statistically associated with radiographic bone erosion during the same period [5].

The critical role of fibroblast-like synoviocytes (FLS), expressing IL-6, MMP-3, and VCAM-1, as a source of biomolecular signaling that precedes clinical manifestations of disease activity. Activated FLS-induced microRNAs, particularly miR-155 and miR-223, were elevated 4.5–6.1-fold in patients with DAS28 > 5.4 and pronounced synovial hyperplasia confirmed by histomorphometric analysis [8].

Of particular interest are data on osteoimmune mediators such as DKK-1 and RANKL. In the study by Gravallese E.M. and Firestein G.S. (2023), the RANKL/OPG ratio in patients with erosive RA exceeded control values by 3.8 times, and elevated DKK-1 levels were strongly correlated with progressive marginal joint erosion ($p < 0.001$). These findings highlight the practical value of including osteoimmunological markers in the comprehensive assessment of a patient's biomolecular profile.

The clinical relevance of emerging immunological markers is also confirmed by regional cohort data. In the study by Ziyadullaev Sh.Kh. et al. (2020) conducted in the Bukhara region, patients with pronounced synovial hyperplasia and CDAI > 22 exhibited VEGF-A levels above 250 pg/mL. This was associated with aggressive angiogenesis and synovial membrane thickening on Doppler ultrasound. Elevated VEGF-A levels positively correlated with IL-17 titers ($r = 0.74$), indicating involvement in the pathogenic cascade of neovascularization and chronic inflammation.

Several studies have focused on stratifying patients based on predictors of response to specific therapeutic targets. In the study by Barouta G. et al. (2017), patients positive for anti-MCV and with IL-6 > 30 pg/mL responded better to tocilizumab therapy, with ACR50 achieved in 64% versus 42% of seronegative patients ($p = 0.012$). Similar findings were reported for leflunomide by Chichasova N.V. et al. (2013), where baseline TNF-α > 28 pg/mL was associated with a 2.7-fold higher risk of nonresponse (OR = 2.72; 95% CI: 1.6–4.3).

With the increasing number of targeted therapies, biomarkers capable of predicting adverse effects have become essential. According to Collins T.R. (2018), patients with low serum BAFF and elevated IFN-γ had a 37% higher risk of serious infectious complications during anti-TNF therapy. This underscores the need for biomonitoring not only to assess efficacy but also to ensure individualized safety in therapy selection.

A multifactorial biomarker interpretation model was developed by Dadoun S. et al. (2013). Using a scoring system that incorporated IL-6, anti-CCP, miR-146a, and HLA-DRB1*04, the authors achieved a stratification accuracy exceeding 82% (AUROC = 0.84), supporting its utility as a prognostic tool in daily clinical practice.

Conclusion

The reviewed evidence confirms the critical role of molecular biomarkers in modern diagnostics and personalized management of rheumatoid arthritis. Traditional inflammatory markers such as CRP and ESR remain useful indicators of disease activity phase but lack the specificity and predictive strength of immunologic and cytokine-based markers. Antibodies to modified proteins—including anti-CCP, anti-MCV, and anti-CarP—are directly associated with erosive and rapidly progressing disease phenotypes, particularly in carriers of HLA-DRB1*04:01 and other immunogenetic risk alleles. Cytokine profiles dominated by IL-6, TNF-α, and IL-17, along with microRNAs (miR-146a, miR-155) and osteoimmune mediators (RANKL, DKK-1, VEGF-A), exhibit strong prognostic value in assessing both disease activity and therapeutic response.

Molecular stratification has been shown to predict the efficacy of both conventional and targeted therapies (methotrexate, tocilizumab, JAK inhibitors), while reducing the incidence of clinical treatment failure. The integration of multi-omics panels, transcriptomic and proteomic approaches into routine clinical practice holds promise for the development of robust clinico-biological algorithms aimed at achieving sustained remission and preventing disabling complications.

In sum, this review highlights the urgent need for the systematic implementation of validated biomarkers in RA clinical decision-making, and supports further multicenter research incorporating molecular platforms in protocols for disease activity assessment and therapeutic monitoring.

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