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REVIEW

Clinical predictors of response to methotrexate in patients with rheumatoid arthritis: A systematic review

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation, structural damage, and disability. Methotrexate (MTX) is the first-line treatment; however, up to one-third of patients exhibit an inadequate response. This systematic review aimed to identify clinical, serological, genetic, pharmacogenomic, and treatment-related predictors of MTX response in RA.

Methods: A systematic search of PubMed/MEDLINE, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL) was conducted for studies published up to May 22, 2024. Randomized controlled trials, prospective cohort studies, and registry-based studies evaluating predictors of MTX efficacy were included. In total, 87 studies met the eligibility criteria and were included in the review. Predictors were categorized as demographic/clinical, disease-related, serological/immunological, genetic/pharmacogenomic, treatment-related, and emerging. Evidence was synthesized narratively, with consideration of methodological quality and consistency.

Results: Consistent predictors of favorable MTX response included lower baseline disease activity, better functional status, early MTX initiation, and absence of erosive disease. Male sex, older age, and moderate alcohol consumption were associated with improved outcomes, although these associations may be influenced by treatment-related and behavioral confounders, whereas smoking and higher body mass index were linked to reduced efficacy. Several inflammatory and cellular biomarkers were associated with MTX response, although individual pharmacogenetic variants showed limited reproducibility. Treatment-related factors, including subcutaneous administration, rapid dose escalation, glucocorticoid co-therapy, and folate supplementation, were associated with improved efficacy and tolerability. Emerging proteomic, epigenetic, and metabolomic signatures demonstrated potential for early response prediction.

Conclusions: Overall, early disease control and optimized treatment strategies appear to be more reliable predictors of MTX response than isolated demographic or genetic factors. Integration of clinical predictors with emerging molecular biomarkers may support personalized treatment approaches and earlier identification of MTX non-responders.

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KEYWORDS
arthritis, rheumatoid; folic acid antagonists; patient outcome assessment; precision medicine; systematic review

Key Points

- Early achievement of clinical response and optimized methotrexate strategy (early initiation, dose escalation, subcutaneous route, folate supplementation) are the most consistent predictors of treatment success, outperforming isolated demographic, serological, or genetic markers.
- This review integrates clinical, immunological, genetic, and multi-omic evidence, highlighting dynamic inflammatory and adenosine-pathway biomarkers and reinforcing treat-to-target principles as the most actionable framework for personalized methotrexate therapy.

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder involving multiple peripheral joints, often leading to joint destruction, and thus giving rise to severe pain, loss of function, and reduced quality of life.[1,2] To minimize severe joint damage leading to functional disability, early diagnosis and treatment are crucial in patients with RA.[2] Current treatment options include intra-articular administration of glucocorticoids, early intervention with conventional disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX), and the use of biological DMARDs (bDMARDs).[1]

MTX remains the preferred first-line conventional disease-modifying antirheumatic drug (csDMARD) for RA[3] and is often maintained in combination with bDMARDs.[4] However, response to MTX is highly variable: approximately one-third of patients fail to achieve an adequate clinical response,[2] and about 30% discontinue treatment within a few years, roughly half due to inefficacy and half due to adverse effects.[5]

Considering the existence of a "therapeutic window of opportunity" in early RA—during which disease activity is more amenable to modification[6]—early identification of patients unlikely to respond to MTX could be crucial to optimize outcomes. Reliable predictors of MTX response would enable clinicians to personalize therapy, avoiding months of ineffective treatment and allowing earlier initiation of biological or targeted synthetic DMARDs (tsDMARDs).[4,7,8]

Despite the large number of studies investigating potential predictors of MTX response, the evidence remains fragmented and inconsistent, with heterogeneous study designs, biomarkers, and clinical endpoints. A comprehensive synthesis of this literature is therefore essential to clarify which predictors show the most robust and reproducible associations, and to guide their translation into clinical practice. Therefore, this systematic review aims to comprehensively evaluate and summarize the current evidence on clinical, serological, genetic, and molecular predictors of methotrexate response in patients with rheumatoid arthritis, in order to identify reliable biomarkers that could guide more personalized therapeutic strategies.

The primary objective of this systematic review is to systematically identify, evaluate, and synthesize evidence on clinical, serological, genetic, and molecular factors associated with response to MTX therapy in adult patients (≥18 years) with RA.

The secondary objective is to provide guidance for optimizing the clinical use of MTX, including early diagnosis and initiation, appropriate selection of the route of administration, and personalized treatment management based on the identified factors.

2 | METHODS

2.1 | Protocol and registration

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[9] The review protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD42023464365).

2.2 | Eligibility criteria

We included randomized controlled trials (RCTs), cohort studies (prospective or retrospective), case–control, and cross-sectional studies assessing the efficacy of MTX in adult patients (≥18 years) diagnosed with RA according to recognized classification criteria. Studies enrolling patients starting MTX for the first time were eligible, regardless of dose or route of administration. To ensure adequate evaluation of treatment response, studies were required to assess outcomes after a minimum treatment duration of 3–6 months following MTX initiation. Concomitant glucocorticoid use was permitted, whereas studies including the initiation of additional DMARDs were excluded.

The main comparison of interest was between responders and non-responders to MTX within the first 6 months of treatment. Eligible outcomes included validated measures of clinical response such as the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) response criteria, Disease Activity Score in 28 joints (DAS28), Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index

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(CDAI), or the Health Assessment Questionnaire (HAQ). All predictors assessed at baseline and quantitatively analyzed in relation to MTX response were considered, including demographic, clinical, serologic, genetic, and other laboratory markers. Studies with a minimum follow-up of 6 months were included. Publications in English, Spanish, or Portuguese were considered.

A summary of the PICO framework used to define the research question is presented in Table 1.

2.3 | Information sources and search strategy

A comprehensive literature search was conducted in PubMed/MEDLINE, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to May 22, 2024, without restrictions on publication status. The search strategy combined controlled vocabulary terms (such as Medical Subject Headings - MeSH) and free-text terms related to rheumatoid arthritis, methotrexate, and predictors of treatment response, using Boolean operators ("AND", "OR") appropriately.

Reference lists of included articles were manually screened to identify additional eligible studies. The search strategy was developed in consultation with a medical librarian experienced in systematic reviews to ensure reproducibility and completeness. Detailed search strategies for all databases are reported in the Supporting information: Supplementary Material A.

TABLE 1 PICO framework for predictors of methotrexate response in rheumatoid arthritis.

PICO	Description	Search terms
Population	Patients with rheumatoid arthritis aged 18 years or over	Arthritis, Rheumatoid[1] OR Rheumatoid arthritis
Intervention	Treatment with methotrexate for the first time	Methotrexate[1] OR Folic acid antagonists[1]
Comparison	Responders versus non-responders	Predictors OR Patient outcome assessment[1] OR Treatment outcome[1]
Outcome(s)	Disease activity scores and their predictors	OR Biomarkers[1] OR Risk factors[1] OR Prognosis[1] OR Response OR Clinical response OR Clinical effectiveness OR Treatment effectiveness OR Treatment efficacy OR Clinical efficacy

[1]Medical subject heading terms.

2.4 | Study selection

All retrieved citations were imported into the Rayyan software to facilitate screening. Two reviewers independently screened titles and abstracts, followed by full-text evaluation of potentially eligible studies. Discrepancies were resolved by consensus or by a third reviewer. Reasons for exclusion were documented at the full-text stage. When multiple publications referred to the same dataset, the most comprehensive or recent report was used.

2.5 | Data extraction

Two reviewers independently extracted data using a standardized form. Extracted variables included study characteristics (design, year, country, sample size), patient demographics (age, sex, disease duration, rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) status), MTX treatment details (dose, route, duration, concomitant therapy), predictors evaluated, and outcomes (measures of response and effect estimates). Disagreements were resolved by discussion or third-party adjudication.

2.6 | Risk of bias assessment

The risk of bias was independently assessed by two reviewers according to study design: the Cochrane Risk of Bias 2.0 tool for RCTs, the Newcastle–Ottawa Scale (NOS) for observational studies, and the AXIS tool for cross-sectional studies. Although the QUIPS tool had been initially planned in the protocol registered in PROSPERO for the assessment of prognostic factor studies, design-specific tools were ultimately used due to the heterogeneity of the included study designs. Each domain was rated as low, moderate, or high risk of bias. For observational studies assessed using the NOS, predefined thresholds based on the overall star rating were applied to identify studies with insufficient methodological quality. For cross-sectional studies assessed with the AXIS tool, studies were considered to have a high risk of bias when major concerns were identified across key methodological domains, including participant selection, outcome definition, measurement validity, or statistical methods. Studies judged to be at high risk of bias were excluded from the final synthesis to ensure the methodological robustness of the review. Risk-of-bias assessments were therefore used both to appraise the methodological quality of eligible studies and to support exclusion of studies considered methodologically inadequate. Consensus was reached by discussion, with a third reviewer involved when necessary.

2.7 | Data synthesis

Given the heterogeneity across study designs, predictors, and outcome measures, a narrative synthesis was performed. Findings were summarized in text and tables

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according to predictor categories (clinical, serologic, genetic, or laboratory). Patterns and consistencies of predictors across studies were explored, highlighting factors repeatedly associated with MTX response. Quantitative pooling was not feasible due to methodological diversity among studies.

2.8 | Ethical considerations

Since this study is based exclusively on data from previously published literature and does not involve human or animal participants, it did not require ethical approval. The findings will be disseminated through publication in a peer-reviewed journal and presentations at scientific conferences.

3 | RESULTS

3.1 | Study selection

The study selection process followed the PRISMA 2020 guidelines and is illustrated in Figure 1. A total of 17,250 records were identified through database searches: 2135 from PubMed/MEDLINE, 12,681 from Scopus, and 2434 from CENTRAL. After the removal of 3114 duplicate records, 14,136 records remained for title and abstract screening. Of these, 13,535 were excluded for not meeting the inclusion criteria. The remaining 601 full-text reports were retrieved for detailed assessment, of which two could not be obtained in full text. A total of 599 reports were assessed for eligibility. Following full-text screening, 453 studies were excluded for the following reasons: wrong outcome ($n = 272$), wrong study design ($n = 50$), wrong publication type ($n = 44$), wrong drug ($n = 30$), inadequate study duration ($n = 26$), foreign language ($n = 23$), wrong population ($n = 6$), and background or narrative article ($n = 2$).

A total of 146 studies initially met the inclusion criteria and were included for quality assessment. After excluding 59 studies due to high risk of bias or insufficient methodological quality, 87 studies were finally included in the qualitative synthesis. An overview of the main characteristics, outcomes, and identified predictors of methotrexate response among the 87 included studies is provided in the Supporting information: Supplementary Material B.

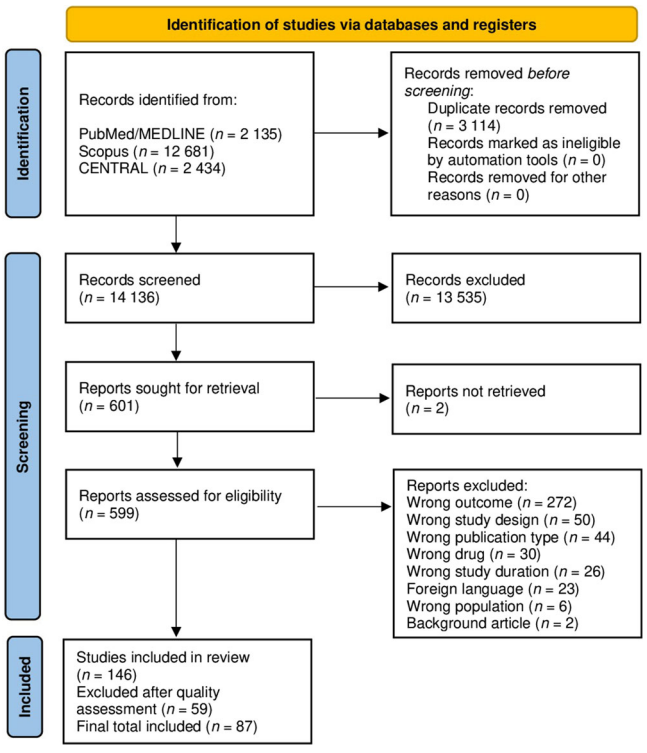

FIGURE 1 PRISMA 2020 flow diagram illustrating the selection process of studies identified through database and register searches. A total of 17,250 records were identified (PubMed/MEDLINE: $n = 2135$; Scopus: $n = 12,681$; CENTRAL: $n = 2434$). After removal of 3114 duplicates, 14,136 records were screened, and 13,535 were excluded. Of the 601 reports sought for retrieval, 2 were not retrieved. A total of 599 reports were assessed for eligibility, with 146 studies included in the review. Following quality assessment, 59 studies were excluded, resulting in a final total of 87 studies included in the systematic review. Adapted from Page et al., *BMJ* 2021;372:n71 (PRISMA 2020 statement).

3.2 | Demographic and clinical predictors

Across RCTs and observational cohorts, few demographic variables consistently predicted MTX response in rheumatoid arthritis. Lower baseline disease activity and male sex emerged as favorable predictors in early RA, whereas age, disease duration, and BMI showed no consistent associations.[10–14] Registry data further supported male sex as a positive predictor, while findings for age were heterogeneous.[15–21] Current smoking and higher BMI were consistently associated with poorer outcomes, whereas moderate alcohol consumption predicted reduced risk of inadequate response.[16,20,22–25] Psychosocial factors, particularly baseline depression and anxiety, substantially reduced the likelihood of remission independent of clinical covariates.[26]

Overall, male sex and moderate alcohol intake favored MTX efficacy, whereas current smoking, elevated BMI, and psychological distress predicted poorer outcomes (Table 2).[15,16,18,20,22,24,26]

3.3 | Disease-related predictors

Evidence from RCTs demonstrates that baseline disease burden is a major determinant of MTX response in rheumatoid arthritis. Lower baseline disease activity and better functional status predicted higher rates of good EULAR responses, while higher composite scores (DAS28, SDAI, CDAI), elevated inflammatory markers, and increased joint counts were associated with poorer outcomes.[10,11,22] Early MTX initiation—particularly within 3 months of symptom onset—significantly improved response likelihood.[13,23] Structural features also influenced prognosis, with baseline erosions and seropositivity predicting radiographic progression despite clinical improvement.[27–29]

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TABLE 2 Demographic, clinical, disease-related, serological/immunological, genetic/pharmacogenomic, emerging, and treatment-related predictors of MTX response.

Category	Specific predictor	Association with MTX response	Evidence summary	References
1. Demographic and Clinical Predictors				
Demographic factors (RCTs)	Baseline disease activity	Lower baseline disease activity predicts higher likelihood of good EULAR response	[Consistent] Initial inflammatory burden strongly influences MTX efficacy	[10]
	Sex	Male sex associated with higher probability of MTX response	[Consistent] Consistent evidence	[10]
	Age	Inconsistent/heterogeneous evidence	[Inconsistent] Conflicting or heterogeneous findings across studies	[10, 12–14]
	Disease duration	No significant association	[Inconsistent] No consistent association observed across studies	[10, 12–14]
	BMI	No significant association	[Inconsistent] No consistent association observed across studies	[10, 12–14]
	Smoking	Inconsistent/heterogeneous evidence	[[Inconsistent] Conflicting or heterogeneous findings across studies	[12–14]
	Comorbidities	Inconsistent/heterogeneous evidence	[Inconsistent] Conflicting or heterogeneous findings across studies	[12–14]
Demographic factors (observational/registry)	Sex	Male sex consistently predicts better remission and EULAR good response	[Inconsistent] Conflicting or heterogeneous findings across studies—	[15–19]
	Age	Variable: younger age sometimes predicts poorer response; older age predicts higher remission	[Inconsistent] Conflicting or heterogeneous findings across studies—	[17, 20, 21]
	Smoking (current)	Consistent evidence for poorer MTX outcomes	[Consistent]; past smoking shows no effect	[16, 20, 22, 23]
	BMI	Higher BMI reduces likelihood of remission	[Consistent] Consistent evidence for reduced response (stronger in established RA)	[24, 25]
	Alcohol intake (≥ 1 unit/week)	Reduced likelihood of inadequate MTX response	[Exploratory] Lifestyle modifier	[22]
Psychosocial factors (prospective multicentre)	Depression and anxiety	Reduce likelihood of remission; worse patient-reported outcomes	[Consistent] OR 0.22–0.48; persists after adjustment	[26]
2. Disease-related Predictors				
Baseline disease activity (RCTs)	Low baseline disease activity	Higher likelihood of achieving a good EULAR response	[Consistent] Lower inflammatory burden improves treatment efficacy	[10]
	High composite disease activity (DAS28, SDAI, CDAI)	Reduced probability of remission or low disease activity	[Consistent] Consistently associated with poorer outcomes	[11, 22]
Inflammatory burden (RCTs)	Elevated acute-phase reactants	Associated with reduced likelihood of remission	[Consistent] Marker of higher baseline inflammation associated with poorer outcomes	[11, 22]
Functional status (RCTs)	Lower HAQ scores	Independently linked to favorable outcomes	[Consistent] Functional status independently predicts treatment response	[22]
Timing of therapy (RCTs)	Early MTX initiation (< 3 months)	Markedly increases likelihood of EULAR good/moderate response	[Consistent] Early treatment initiation consistently improves outcomes	[13, 23]
Structural features (RCTs)	Baseline erosions	Predict radiographic progression despite clinical improvement	[Consistent] Structural damage consistently associated with poorer response	[27–29]
Structural features (RCTs)	Seropositivity (RF/ACPA)	Predicts radiographic progression	[Consistent] Independent prognostic factor across studies	[27–29]

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TABLE 2 (Continued)

Category	Specific predictor	Association with MTX response	Evidence summary	References
Baseline activity (observational)	High DAS28, SDAI, CDAI	Predict poorer MTX efficacy	[Consistent] Findings consistently replicated in observational cohorts	[20, 25, 30–32]
Inflammatory markers (observational)	Elevated CRP or ESR	Predict poorer outcomes	[Consistent] Independently associated with outcomes across multiple cohorts	[20, 25, 30–32]
Functional/clinical status	High HAQ, tenderness, high PhGA	Associated with MTX non-response	[Consistent] Functional impairment consistently associated with poorer outcomes	[5, 33]
Structural features (observational)	Erosive disease	Predicts poorer outcomes	Consistent evidence across multiple cohorts	[34–36]
Structural features (observational)	Elevated ESR	Predicts worse outcomes	[Inconsistent] Mostly consistent findings with minor conflicting evidence	[34–36, 51]
Pain/assessment factors	Persistent pain; global assessment discordance	Linked to poorer improvement	[Exploratory] Suggests non-inflammatory mechanisms with limited validation	[37]
Imaging predictors	Baseline ultrasound synovitis	Does not improve predictive accuracy	[Consistent] No additional predictive value beyond established clinical variables	[38]
3. Serological and Immunological Predictor				
Classical autoantibodies (RCTs)	ACPA positivity	Not predictive of differential MTX response; associated with radiographic progression and reduced sustained remission	[Consistent] Consistently associated with prognosis but not predictive of treatment response	[27]
	RF status	Inconsistent prediction of MTX response	[Inconsistent] Conflicting evidence with limited predictive utility	[5, 38, 62, 63]
Inflammatory markers (RCTs)	Baseline CRP, ESR	Correlate with MTX response	[Consistent] Higher inflammatory burden consistently associated with reduced efficacy	[40, 41]
	Early CRP reduction; CRP metabolite	Predict subsequent DAS28 improvement	[Exploratory] Dynamic biomarker with emerging predictive relevance	[40, 41]
Matrix remodeling markers (RCTs)	MMP-3	Parallels clinical improvement	[Exploratory] Reflects tissue inflammation with emerging clinical relevance	[40, 41]
	CRP metabolite, C3M, C4M	Declines parallel disease improvement	[Exploratory] Biomarkers of extracellular matrix turnover with emerging evidence	[40, 41]
Composite biomarker panels (RCTs)	MBDA score	Tracks disease activity in MTX-based regimens	[Consistent] Validated composite index reflecting disease activity	[42, 43]
MicroRNA biomarkers (RCTs)	miR-27a-3p	Associated with remission (in combination therapy)	[Exploratory] Evidence from limited cohorts requiring validation	[44]
Inflammatory markers (observational)	Elevated baseline CRP, ESR	Predict inadequate response	Consistent evidence across cohorts	[32, 39]
Autoantibodies (observational)	Anti-SSA antibodies	Associated with reduced likelihood of low disease activity and more residual pain	[Exploratory] Suggests immune-mediated modulation with limited evidence	[37]
Chemokines (observational)	CXCL13	Higher baseline levels predict remission	[Exploratory] Mechanistic biomarker with emerging predictive relevance	[45]
Metabolic markers (observational)	Adiponectin	Unrelated to clinical improvement	[Exploratory] Reflects metabolic processes rather than direct disease prediction	[46]
Immune-cell signatures (observational)	Circulating monocytes (CD14+highCD16−/+)	Predict non-response	[Exploratory] Immune signature associated with response; limited validation	[47]

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TABLE 2 (Continued)

Category	Specific predictor	Association with MTX response	Evidence summary	References
	Regulatory B cells (CD19 + CD24^hiCD38^hi)	Identify responders	[Exploratory] Immunoregulatory mechanism with emerging evidence	[7, 48]
	Soluble CD14	Lower levels in responders	[Exploratory] Mechanistic biomarker requiring further validation	[7, 48]
	High peripheral lymphocyte count	Predict inadequate response	[Exploratory] Associated with immune dysregulation; limited predictive validation	[39]
Adenosine pathway (mechanistic)	CD39 expression on T cells	Higher expression associated with lower DAS28 and favorable outcomes	[Exploratory] Mechanistic pathway associated with MTX response	[49]
	CD39+ Tregs; *AMPD1*, *ADORA2B*, *ADORA3*	Predict remission; outperform baseline DAS28	[Exploratory] Mechanistic biomarkers with emerging predictive value	[50]
Emerging protein biomarkers	IgG Fc glycosylation (low FA2/[FA2G1 + FA2G2])	Identifies MTX non-responders	[Exploratory] Novel biomarker signature requiring validation	[90]
	Calprotectin (S100A8/9); tumor necrosis factor-α	Superior to CRP in tracking response	[Consistent] Sensitive markers of inflammation correlated with response	[51, 52]
Novel ACPA specificities	Anti-citrullinated fibrinogen peptide antibodies	Predict clinical improvement	[Exploratory] Emerging autoantibody specificity with limited evidence	[53]
Genetic/epigenetic markers	Global DNA methylation	Associated with differential response	[Exploratory] Epigenetic mechanisms associated with response	[54]
	Soluble *HLA-G* genotypes	Associated with tolerogenic responses	[Exploratory] Genetic modulation of immune tolerance	[55]
Metabolomic markers	Metabolomic profiles	Discriminate responders	[Exploratory] Systems-level biomarker requiring validation	[56]
Glucocorticoid pathway	Glucocorticoid receptor α isoforms A/B induction	Correlates with improvement	[Exploratory] Mechanistic interaction with emerging relevance	[57]
T-cell phenotypes	Naïve CD4 + T cells	Predict remission on MTX monotherapy	[Exploratory] Immune phenotype associated with response; limited validation	[58]
	IL-13 + CD4 + Th2-skewed effector-memory cells	Identify non-responders	[Exploratory] Immune skewing linked to poor response; exploratory evidence	[59]
Synovial cell assays	MTX-induced synoviocyte apoptosis	Mirrors clinical sensitivity	[Exploratory] Experimental correlate of clinical response	[60]
Pharmacogenetic models	RF + clinical activity + folate-pathway variants	Improve prediction of MTX efficacy	[Exploratory] Composite model with emerging predictive performance	[16, 61]
4. Genetic Pharmacogenomic Predictors				
Folate pathway (RCTs)	*MTHFR* 1298AA; *MTHFR* 677CC	Better clinical improvement; reduced toxicity	[Consistent] Genetic variants consistently modulate MTX efficacy/ toxicityin BeSt trial	[16]
	MTHFR 1298 C allele	Increased adverse events	[Consistent] Consistent association with adverse events	[16]
HLA & immune genes (RCTs)	*HLA-DRB1* shared epitope	Minimal short-term prediction; post hoc: better ACR and LDA in ADA + MTX arm	[Exploratory] Signal observed only in post hoc analyses	[11, 64]
Immune receptors (RCTs)	*FCGR2B* rs1050501	Predicts remission	[Exploratory] Association observed in secondary analyses	[64]
Cytokine receptor (RCTs)	*IL4R* rs1805010	Correlates with radiographic progression in MTX monotherapy	[Inconsistent] Limited and inconsistent predictive value	[11, 64]

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TABLE 2 (Continued)

Category	Specific predictor	Association with MTX response	Evidence summary	References
Folate pathway (cohorts)	*MTHFR* 677CC	Faster and greater improvement	[Exploratory] Association observed in a single cohort (Polish study), requiring replication in independent populations	[65]
	MTHFR 677T; *GGH* –401CC	Increased adverse events	[Exploratory] Genetic variants associated with MTX toxicity in limited cohort data; requires external validation	[65]
Transport pathway (cohorts)	*RFC1*/*SLC19A1* 80AA	Hepatotoxicity, alopecia	[Exploratory] Genotype associated with MTX toxicity in cohort data; limited replication and requires external validation	[65]
Folate pathway (Italian cohort)	*MTHFR* C677T, A1298C	No significant associations	[Inconsistent] Effect modified or not reproducible across conditions	[66]
Chromatin signature	5-locus conformation signature (*IFNAR1*, *IL21R*, *IL23*, *CXCL13*, *IL17A*)	Predicts non-responders with high negative predictive value	[Exploratory] High predictive accuracy but limited external validation	[67]
Transport & purine pathway	*RFC1* G80A	Predicts need for biologic escalation	[Exploratory] Retrospective findings requiring prospective validation	[68]
Purine metabolism (Malaysia)	*ATIC* rs2372536, rs4673993	Better MTX response	[Exploratory] Population-specific findings requiring replication	[69]
Candidate genes	*MTHFR* rs17421511; *DHFR* rs1643650; *ATIC* rs16853826	Modulate efficacy/toxicity	[Exploratory] Evidence from limited targeted studies	[70]
NK cell receptors	*KIR2DS4* full-length genotype	Predicts poor MTX response	[Exploratory] Association observed independent of HLA-C ligand status; limited cohort evidence requiring replication	[34]
Transporters (multivariable)	*ABCB1*, *ABCC3*	Improve prediction beyond clinical variables	[Exploratory] Multivariable model shows moderate predictive performance (AUC 0.75); requires external validation	[25]
Metabolism & transport	*ATIC* C347G; *RFC1* G80A; *ITPA* C94A	Associated with differential efficacy	[Exploratory] Context-dependent associations (sex- and ACPA-dependent) with limited reproducibility across studies	[17]
Transporters	*SLC19A1* –43T > C; *SLC04A1*, *SLC22A2*, *SLC28A2*	Not independently predictive	[Inconsistent] No consistent predictive association	[92, 93]
Composite indices	Clinical–pharmacogenetic index	Moderate internal accuracy but failed external validation	[Inconsistent] Limited reproducibility across cohorts	[36]
Japanese cohort	*TYMS* 3'-UTR; MTX-polyglutamate levels; ESR	Predicted response	[Exploratory] Composite predictive model with limited validation	[31]
Reproducible locus	*MTRR* rs1801394	Consistently predicts poor response	[Consistent] Reproducible association across independent cohorts	[71]
Folate/methionine pathway (ARCTIC-SNPs)	*MTRR* rs1801394; *DHFR* rs408626; *AIF-1* rs2259571	Associated with continuous disease activity measures	[Exploratory] Association with disease activity metrics; limited validation	[72]
Folate pathway (ARCTIC-SNPs)	*MTHFR* rs1801133	Improved fatigue	[Exploratory] Association with secondary outcomes only	[72]
Transport/pathway genes (ARCTIC-SNPs, uncorrected)	*MTHFR* rs1476413; *ABCC1* rs3784864; *SLC19A1* rs2274808; *AIF-1* rs2259571	Associated with non-remission and MTX monotherapy failure	[Inconsistent] Association not robust after correction	[72]
GWAS/pharmacogenomic panel	Seven-SNP model via 1966-variant array	Predicts MTX response (AUC 0.82–0.84)	[Exploratory] Predictive value improved in composite models	[73]

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TABLE 2 (Continued)

Category	Specific predictor	Association with MTX response	Evidence summary	References
Machine learning (exome)	Functional haplotypes + 5 clinical predictors	AUC 0.78–0.83	[Exploratory] Machine-learning model with emerging evidence	[74]
Composite metabolic/immune	*AMPD1*, *ATIC*, *ITPA*, *MTHFD1* + clinical predictors	AUC 0.85; accurate responder classification	[Consistent] Validated composite model, though limited clinical applicability	[16, 75]
DNA methylation	Four CpG sites; global methylation (LINE-1)	Predict 6-month outcomes; interaction with serostatus	[Exploratory] Epigenetic predictors requiring validation	[76, 77]
Drug retention genes	*FPGS* expression	Reduced expression predicts non-response	Exploratory] Mechanistic association with limited clinical validation	[54]
Immune regulation	*HLA-G* polymorphisms	Associated with immune tolerance	[Exploratory] Mechanistic role in immune tolerance	[55]
Folate cycle enzymes	*FPGS*, *GGH* transient effects	Short-term modulation of MTX response	[Exploratory] Transient effects with unclear long-term predictive value	[78]
5. Emerging Predictors				
Imaging biomarkers	Power Doppler ultrasound	Tracks deep synovitis resolution; Power Doppler negativity associated with remission	[Consistent] Prednisone + MTX improved PD outcomes at 12 months compared with MTX alone	[28]
Proteomic biomarkers	MBDA score	Mirrors DAS28; faster improvements with prednisone + MTX	[Consistent] More MBDA proteins changed under combination therapy	[44]
	Extracellular matrix neo-epitopes (C3M, C4M)	Early reductions predict later DAS28 improvement; discriminate ACR50 responders	[Consistent] Early biomarker changes consistently predict subsequent clinical response in MTX-based regimens (AMBITION)	[41]
	MMP-3	Declines with disease activity; correlates with DAS28 under MTX	[Consistent] Biomarker consistently correlates with disease activity and treatment response	[40]
MicroRNA biomarkers	miR-27a-3p	Predicts Boolean remission at 12 months in ADA + MTX	[Exploratory] Therapy-specific microRNA signature identified in a substudy; requires validation in independent cohorts	[42]
Composite imaging/proteomic conclusion	Power Doppler, MBDA proteins, C3M/C4M, MMP-3	Most robust dynamic early predictors	[Consistent] Multiple biomarkers consistently demonstrate early predictive value across MTX-based treatment strategies	[28, 40–42, 44]
Pharmacometabolomic markers	MTX-polyglutamates (MTX-polyglutamates)	PG ≥ 83 nmol/L predicts improvement; >105–130 nmol/L increases hepatotoxicity	[Consistent] Dose–response relationship consistently observed, with defined therapeutic window influenced by clinical factors (e.g., BMI, albumin)	[82]
	Adiponectin	Increases under MTX ± steroids but unrelated to response	[Inconsistent] No consistent association with treatment response; reflects metabolic changes rather than predictive utility	[46]
Adenosine pathway markers	CD39 expression on CD4 + CD25^high Tregs	High baseline expression predicts 6-month remission	[Exploratory] Mechanistic biomarker associated with MTX response; evidence from limited cohorts requiring external validation	[49]
	AMPD1, *ADORA2B*, *ADORA3* transcription	High expression predicts remission (AUC 0.92 vs 0.67 for DAS28)	[Exploratory] High predictive performance in mechanistic studies; requires replication in independent cohorts	[50]
Chromatin conformation signatures	*IFNAR1*, *IL21R*, *IL23*, *CXCL13*, *IL17A*	Identifies non-responders with approximately 90% negative predictive value	[Exploratory] High predictive performance with internal validation; requires external validation in independent cohorts	[67]

(Continues)

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TABLE 2 (Continued)

Category	Specific predictor	Association with MTX response	Evidence summary	References
Functional cellular assays	MTX-induced synoviocyte apoptosis	Occurs only in responders	[Exploratory] Ex vivo mechanistic correlate of MTX sensitivity with limited clinical validation	[60]
	Glucocorticoid receptor α isoform A/B induction	Correlates strongly with ACR improvement ($r = 0.92$)	[Exploratory] Strong mechanistic association observed in limited datasets; requires external validation	[57]
Immune-cell phenotypes	CD14^highCD16$^{-/+}$ monocytes	Higher counts predict non-response	[Exploratory] Innate immune signature associated with non-response; limited replication across cohorts	[47]
	Regulatory transitional B cells (CD19 + CD24^hiCD38^hi)	Predict good EULAR response	[Exploratory] Immunoregulatory B-cell subset associated with response; requires validation in independent cohorts	[48]
Soluble immune markers	CXCL13	Higher baseline levels predict remission	[Exploratory] Biomarker associated with remission and synovial inflammation; evidence from limited cohorts requiring validation	[45]
	Soluble CD14	Lower baseline levels identify responders	[Exploratory] Mechanistic association	[7]
Metabolomic profiling	^{1}H-NMR serum metabolites (11-metabolite panel)	Distinguishes responders vs non-responders	[Exploratory] Predictive performance in limited datasets	[56]
Lifestyle factors	Caffeine exposure	No impact on MTX efficacy	[Inconsistent] No consistent effect observed	[86]
Epigenetic markers	Week-4 methylation dynamics	Predict 6-month outcomes	[Exploratory] Early predictive biomarker requiring validation	[76]
	Cell-type-specific methylation patterns	Differentiate responders vs non-responders	[Exploratory] Improved predictive signal in refined models	[89]
	Global LINE-1 methylation	Predictive depending on serostatus	[Exploratory] Context-dependent predictive value; hypermethylation predicts response in seropositive; hypomethylation in seronegative RA	[54]
Glycomic markers	IgG1 Fc galactosylation (FA2/[FA2G1 + FA2G2])	Low ratio identifies non-responders (~70% sensitivity/specificity)	[Exploratory] Moderate predictive performance	[90]
Transcriptomic markers	Whole-blood transcriptomics	Non-responders upregulate CCL4 and anti-apoptotic genes	[Exploratory] Mechanistic insight with limited clinical applicability	[75]
Lipidomics	Serum lipidomic profiling	Poor predictive performance (AUC ~ 0.61)	[Inconsistent] Weak and inconsistent predictive value	[91]
6. Treatment Related Predictors				
Route of administration (RCTs)	Subcutaneous MTX 15 mg/week versus oral	Superior ACR20/70 responses with similar tolerability	[Consistent] Superior efficacy demonstrated in RCTs	[80]
Glucocorticoid co-therapy (RCTs)	Prednisone 10 mg/day (CAMERA-II)	Reduced radiographic progression; faster/deeper responses; higher ACR50/70; earlier sustained remission	[Consistent] Consistent biomarker improvement	[13, 44, 81]
	Prednisone 6.25 mg/day (step-up MTX)	Increased clinical remission and improved synovitis	[Consistent] Safety profile consistent across studies	[28]
Combination therapy (RCTs)	Adalimumab + MTX (OPTIMA)	Higher rates of stable LDA by week 26; superior functional and radiographic outcomes	[Consistent] Improved disease control across trials	[11, 14]
Folate supplementation (RCTs)	Folic or folinic acid	Halved MTX discontinuation; allowed higher dose escalation	[Consistent] Consistently reduces toxicity	[10, 12]
Metabolic markers	Adiponectin	Unrelated to clinical response	[Inconsistent] No consistent association with response	[46]

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TABLE 2 (Continued)

Category	Specific predictor	Association with MTX response	Evidence summary	References
Real-world administration	Initial subcutaneous MTX	Fewer treatment changes; better 1-year control	[Consistent] Improved outcomes in real-world settings	[83]
Dose intensity	≥10 mg/week early escalation; ≥20 mg/week or ≥0.3 mg/kg by 6 months	4× higher 1-year remission and normal function	[Consistent] Dose escalation consistently improves outcomes	[84]
Treat-to-target	2010 criteria-driven strategies	Higher remission rates	[Consistent] Treat-to-target strategies consistently effective	[85]
Lifestyle	Caffeine intake	No effect on MTX efficacy	[Inconsistent] No consistent effect on MTX efficacy	[86]
Japanese cohorts	Early MTX initiation (<3 months)	Higher remission	[Consistent] Early initiation consistently improves outcomes	[23, 33]
	Step-up regimen (8 → 12–16 mg/week)	Superior clinical and radiographic outcomes	[Consistent] Higher doses associated with better outcomes	[31, 87]
	SC MTX	Better response; less GI toxicity	Consistent evidence	[88]
	Elderly patients	Comparable efficacy	[Consistent] Comparable efficacy across age groups	[21]

Note: The table summarizes individual predictors, their reported associations with MTX treatment response, and the corresponding evidence from the literature. Evidence level was qualitatively classified as: [Consistent] (replicated across multiple independent studies or cohorts), [Inconsistent] (conflicting or heterogeneous findings or lack of reproducibility), and [Exploratory] (emerging evidence from limited cohorts, mechanistic studies, or high-dimensional analyses). Labels were standardized within the Evidence Summary column to facilitate rapid interpretation. *ABCB1*, adenosine triphosphate-binding cassette subfamily B member 1; ACR, American College of Rheumatology; *ADORA2B*, adenosine A2B receptor; *AMPD1*, adenosine monophosphate deaminase 1; *AIF-1*, allograft inflammatory factor 1; *ATIC*, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; AUC, area under the receiver operating characteristic curve; CDAI, clinical disease activity index; CRP, C-reactive protein; *CXCL13*, C-X-C motif chemokine ligand 13; DAS28, disease activity score in 28 joints; *DHFR*, dihydrofolate reductase; EULAR, European League Against Rheumatism; *FPGS*, folylpolyglutamate synthase; *GGH*, gamma-glutamyl hydrolase; HAQ, health assessment questionnaire; *HLA*, human leukocyte antigen; *IFNAR1*, interferon alpha/beta receptor subunit 1; *IL4R*, interleukin 4 receptor; *ITPA*, inosine triphosphatase; *KIR2DS4*, killer cell immunoglobulin-like receptor 2DS4; LINE-1, long interspersed nuclear element-1; MBDA, multi-biomarker disease activity; MMP-3: matrix metalloproteinase-3; *MTHFD1*, methylenetetrahydrofolate dehydrogenase 1; *MTHFR*, methylenetetrahydrofolate reductase; *MTRR*, methionine synthase reductase; MTX, methotrexate; RCTs, randomized controlled trials; *RFC1*, reduced folate carrier 1; SDAI, simplified disease activity index, SLC19A1, solute carrier family 19 member 1; *TYMS*, thymidylate synthetase.

Observational and registry studies corroborate these findings: high baseline disease activity, elevated C-reactive protein (CRP)/ESR, greater disability (HAQ), increased tenderness, and physician global assessment consistently predicted non-response, while erosive disease further worsened outcomes.[20,25,30–36] Persistent pain and patient–physician global discordance also signaled reduced clinical improvement.[37] Notably, adding ultrasound synovitis scores did not improve predictive performance beyond traditional clinical measures.[38]

Overall, greater baseline inflammatory and functional impairment, elevated ESR/CRP, and structural damage predict suboptimal MTX response, whereas early disease suppression and absence of erosions identify patients most likely to benefit (Table 2).[5,22,30,33,39]

3.4 | Serological and immunological markers

Across RCTs, several serological and immunological markers were associated with MTX response. RF and ACPA showed inconsistent predictive value: ACPA positivity did not influence short-term clinical response but was linked to greater radiographic progression and reduced sustained remission, indicating primarily prognostic relevance.[27] Baseline CRP and ESR correlated with MTX outcomes, and early reductions in CRP, CRP metabolite, and extracellular matrix turnover markers (C3M, C4M) predicted subsequent DAS28 improvement.[40,41] Matrix metalloproteinase (MMP)–3 levels and multi-biomarker disease activity (MBDA) scores also tracked clinical response.[40–43] Exploratory RCT data suggested that selected plasma microRNAs may act as remission biomarkers, though mainly in combination therapy.[44]

Observational studies corroborated these associations: elevated baseline CRP and ESR predicted inadequate response[32,39]; anti-SSA antibodies were linked to lower likelihood of low disease activity and greater residual pain,[37] and higher baseline C-X-C motif chemokine ligand 13 (CXCL13) levels associated with greater remission rates.[45] Adiponectin levels rose after MTX treatment but were unrelated to clinical improvement, suggesting metabolic rather than disease-specific effects.[46] Additional biomarkers have also been described: elevated circulating monocyte counts predicted MTX non-response,[47] whereas higher frequencies of regulatory B cells and lower levels of soluble CD14 (sCD14) were associated with treatment response.[7,48] High lymphocyte counts also predicted poor response.[39]

Mechanistic and cellular data highlighted a central role for the adenosine pathway. Increased CD39 expression on T cells, higher baseline $CD39^+$ regulatory T cells, and upregulation of adenosine-related genes (*AMPD1*, adenosine monophosphate deaminase 1 [*ADORA2B*], adenosine A2B receptor [*ADORA3*]) predicted favorable outcomes, outperforming baseline DAS28.[49,50] Beyond autoantibodies, emerging

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serological markers included IgG Fc glycosylation ratios identifying non-responders, as well as calprotectin and tumor necrosis factor-α, both superior to CRP in tracking response.[51,52] Additional candidates included anti-citrullinated fibrinogen peptide antibodies,[53] global DNA methylation patterns,[54] soluble human leukocyte antigen (HLA)-G genotypes,[55] metabolomic signatures,[56] glucocorticoid receptor α isoforms A/B induction,[57] naïve $CD4^+$ T-cell frequencies,[58] Th2-skewed interleukin (IL)–13^+CD4^+ T-cell profiles,[59] and MTX-induced synoviocyte apoptosis.[60]

Clinical–pharmacogenetic models incorporating serology, disease activity, and folate-pathway variants enhanced predictive accuracy,[16,61] although RF and ACPA alone did not reliably stratify outcomes across cohorts.[5,38,62,63] Overall, dynamic inflammatory markers, immune-regulatory signatures, and adenosine-pathway activity emerged as the most informative predictors of MTX response (Table 2).

3.5 | Genetic and pharmacogenomic predictors

Across RCTs and pharmacogenetic substudies, genetic influences on MTX response in RA were modest but detectable. Folate-pathway polymorphisms (e.g., methylenetetrahydrofolate reductase [*MTHFR*] 1298AA/677CC) were associated with improved clinical outcomes, whereas the *MTHFR* 1298C allele increased toxicity.[16] In OPTIMA, early disease activity was a stronger predictor of response than *HLA-DRB1*, *IL4R* rs1805010, or *FCGR2B* rs1050501, although post hoc analyses linked shared-epitope copy number to improved ACR responses in the adalimumab+MTX arm and identified *FCGR2B* rs1050501 and *IL4R* rs1805010 as predictors of remission or radiographic progression.[64] Overall, *HLA-DRB1*, *FCGR2B*, *IL4R*, and *MTHFR* variants showed limited predictive capacity compared with early on-treatment response.

Observational cohorts further supported modest genetic effects. *MTHFR* 677CC improved outcomes, whereas *MTHFR* 677T, gamma-glutamyl hydrolase (*GGH*) –401CC, and reduced folate carrier 1 (*RFC1*) 80AA increased toxicity.[65] Results for *MTHFR* variants were inconsistent across populations.[66] A five-locus chromosome conformation signature (interferon alpha/beta receptor subunit 1 [*IFNAR1*], *IL21R*, *IL23*, *CXCL13*, *IL17A*) showed high negative predictive value for identifying non-responders.[67] Additional associations included RFC1 G80A with biologic escalation,[68] 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (*ATIC*) rs2372536/rs4673993 with improved outcomes in Malay patients,[69] and effects of *MTHFR* rs17421511, dihydrofolate reductase (*DHFR*) rs1643650, *ATIC* rs16853826, and killer cell immunoglobulin-like receptor 2DS4 (*KIR2DS4*) genotypes on efficacy or toxicity.[34,70]

Multivariable studies demonstrated incremental improvements when pharmacogenetics were added to clinical prediction. Transporter polymorphisms (*ABCB1*, *ABCC3*) improved model discrimination[65]; interactions among *ATIC* C347G, *RFC1* G80A, and inosine triphosphatase (*ITPA*) C94A were associated with variable efficacy[17]; and *TYMS* 3′-UTR variants, MTX-polyglutamate levels, and ESR contributed to prediction in a Japanese cohort.[31] *MTRR* rs1801394 emerged as one of the few replicable predictors of poorer response.[71]

Recent data from the ARCTIC-SNPs study reinforced the role of folate/methionine pathway genes, identifying associations for *MTRR* rs1801394, *DHFR* rs408626, allograft inflammatory factor 1 (*AIF-1*) rs2259571, and *MTHFR* rs1801133, with additional uncorrected signals in *MTHFR*, adenosine triphosphate-binding cassette subfamily B member 1 (*ABCC1*), solute carrier family 19 member 1 (*SLC19A1*), and *AIF-1* variants.[72] Genome-wide and machine-learning approaches achieved areas under the receiver operating characteristic curves (AUCs) of 0.78–0.84,[73,74] consistent with earlier composite models integrating folate and purine variants (*AMPD1*, *ATIC*, *ITPA*, methylenetetrahydrofolate dehydrogenase 1 [*MTHFD1*]) with clinical predictors.[16,75]

Epigenetic studies showed that early differential methylation predicted 6-month outcomes,[76] long interspersed nuclear element (LINE)–1 methylation interacted with serostatus,[77] and reduced folylpolyglutamate synthase (*FPGS*) expression was associated with poor response.[54] Additional contributions arose from *HLA-G* polymorphisms[55] and transient *FPGS*/*GGH* effects.[78]

Overall, few single genetic variants show consistent predictive value. Modest, population-dependent effects are observed across folate, purine, transport, and immune-regulatory pathways (e.g., *MTHFR*, *RFC1*/*SLC19A1*, *ATIC*, *TYMS*, *MTRR*). Predictive accuracy improves when genetic, clinical, and biochemical markers are combined, with multi-omic and machine-learning frameworks emerging as the most promising strategies for individualized MTX therapy (Table 2).[67,71–75,79]

3.6 | Treatment-related predictors

Across RCTs, treatment-related factors consistently influenced MTX efficacy (Table 2). The only head-to-head trial showed that subcutaneous MTX (15 mg/week) produced superior ACR20/70 responses versus the same oral dose, with particular benefit in patients with longer disease duration and in oral non-responders who switched to the subcutaneous route.[80] Concomitant low-dose glucocorticoids improved early and sustained outcomes: in CAMERA-II, prednisone co-therapy accelerated remission, enhanced radiographic protection, and produced larger biomarker reductions without excess toxicity.[13,44,81] Additional studies confirmed that adding low-dose prednisone enhanced clinical and ultrasound improvement compared with MTX monotherapy.[28] Combination therapy also modulated outcomes: in OPTIMA, adalimumab+MTX doubled stable low disease activity versus MTX alone (44% vs. 24%),[11] and step-up addition of adalimumab later achieved similar clinical outcomes despite slightly greater radiographic progression.[14] Folate supplementation reduced

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MTX discontinuations, enabled higher dosing, and predicted successful escalation, whereas lack of folate, higher BMI, prior gastrointestinal intolerance, and female sex were associated with MTX withdrawal.[10–12]

Observational and pharmacokinetic data further supported these RCT findings. Early erythrocyte MTX-polyglutamate levels predicted subsequent DAS28 improvement, although toxicity increased above specific MTX-PG thresholds.[82] BMI and serum albumin influenced this therapeutic window. Adiponectin changes did not correlate with response.[46] Pharmacogenetic variants such as *RFC1* 80GG, *MTHFR* 677T, and *GGH* –401CC were associated with higher toxicity or need for biologic co-therapy.[65,68] Real-world cohorts confirmed that subcutaneous initiation reduced treatment changes and improved control,[83] and that higher dose-intensity regimens (≥10 mg/week by 3 months, escalating to ≥20 mg/week by 6 months) markedly increased remission likelihood.[84] Early disease activity suppression (first 12 weeks) remained a consistent predictor of long-term response across regimens.[30] Intensive, guideline-driven strategies achieved higher remission rates than less structured approaches.[85]

Patient-level modifiers also influenced outcomes. Higher BMI reduced remission despite higher prescribed MTX doses.[24] Sex-related prescribing differences affected treatment exposure but not efficacy.[19] Autoantibody dynamics reflected overall immunosuppression rather than MTX-specific prediction.[63] Caffeine intake had no detrimental effect on MTX response.[86] Predictive models integrating baseline DAS28, HAQ, BMI, smoking, erythrocyte folate, and optional genetic data achieved AUC ≈ 0.75 for predicting insufficient response,[25] while machine-learning models highlighted high lymphocyte counts and corticosteroid co-therapy as markers of inadequate response (AUC 0.72).[39]

Japanese inception cohorts reinforced key principles: MTX initiation within 3 months significantly improved remission[23,33]; step-up regimens to 12–16 mg/week outperformed lower fixed doses[31,87]; subcutaneous dosing improved efficacy and reduced gastrointestinal events relative to oral MTX[88]; and efficacy was maintained in elderly patients.[21] Higher baseline DAS28, smoking, and absence of alcohol use predicted MTX non-response,[22] while ultrasound synovitis scores did not enhance prediction.[38]

Overall, early initiation, adequate dose escalation, subcutaneous administration, glucocorticoid co-therapy, and folate supplementation consistently predicted better MTX outcomes, whereas high BMI, persistent disease activity at 3 months, and select pharmacogenetic variants identified patients likely to require treatment intensification.[21–23,31,33,38,87,88]

3.7 | Emerging predictors

Across RCTs and translational studies, several emerging biomarkers showed utility for anticipating or monitoring MTX response, although most function as adjunctive rather than standalone predictors (Table 2). Imaging and proteomic markers—particularly Power Doppler ultrasound, MBDA proteins, extracellular matrix neoepitopes (C3M/C4M), and MMP-3—consistently tracked treatment trajectories. Low-dose prednisone added to MTX improved both clinical remission and Power Doppler negativity at 12 months.[28] In CAMERA-II, MBDA scores paralleled DAS28 and captured faster early improvements under combination therapy.[44] Early reductions in C3M/C4M discriminated ACR50 responders from non-responders,[41] while MMP-3 remained specifically correlated with DAS28 in MTX-treated patients.[40] A microRNA substudy identified miR-27a-3p as a predictor of Boolean remission, although only in adalimumab+MTX recipients.[42]

Pharmacometabolomic and mechanistic indicators also provided predictive value. Erythrocyte MTX-polyglutamate levels at week 12 predicted DAS28 improvement, whereas higher levels (>105–130 nmol/L) increased hepatotoxicity.[82] Adiponectin changes reflected drug exposure but not efficacy.[46] Indicators of adenosine-pathway competence—higher $CD39^+$ regulatory T-cell expression and upregulation of *AMPD1*, *ADORA2B*, and *ADORA3*—strongly predicted remission (AUC 0.92).[49,50] A chromosome conformation signature involving *IFNAR1*, *IL21R*, *IL23*, *CXCL13*, and *IL17A* identified non-responders with approximately 90% negative predictive value.[67] Functional assays showed that MTX-induced synoviocyte apoptosis occurred only in samples from clinical responders,[60] and glucocorticoid receptor α isoform A/B induction correlated tightly with ACR improvement ($r = 0.92$).[57]

Immune and soluble markers offered additional mechanistic insights. Elevated baseline monocyte subsets predicted non-response,[47] whereas higher regulatory B-cell frequencies predicted EULAR response.[48] CXCL13 predicted remission after MTX-based therapy,[45] and low sCD14 levels identified responders.[7] Metabolomic profiling distinguished responders from non-responders,[56] and caffeine intake showed no adverse interaction with MTX.[86]

Multi-omic and molecular signatures further refined the prediction. Early DNA methylation changes (Week 4) predicted 6-month outcomes,[76] and cell-specific methylation patterns differentiated responders from non-responders.[89] Baseline LINE-1 methylation interacted with serostatus in predicting response.[54] Low IgG1 Fc galactosylation identified non-responders with approximately 70% accuracy,[90] and anti-citrullinated fibrinogen peptide antibodies independently predicted DAS28 and ACR outcomes.[53] Transcriptomic profiling showed that non-responders upregulated chemokine and anti-apoptotic pathways,[75] whereas lipidomics showed limited predictive performance.[91]

Together, early dynamic imaging/proteomic markers, MTX-polyglutamate levels and adenosine-pathway metrics, and multi-omic signatures (chromatin conformation, DNA methylation, glycomics, transcriptomics, and immunophenotyping) represent the most promising emerging predictors of MTX response, particularly when integrated with clinical variables.[7,28,40–42,44–50,53–57,60,67,75,76,82,86,89–91]

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4 | DISCUSSION

Across study designs, two themes emerged as the most dependable determinants of MTX response in RA: the burden of inflammation at baseline and—more decisively—its early suppression under therapy. RCTs consistently showed that lower baseline disease activity and better function, as well as the absence of erosions, are associated with subsequent clinical success on MTX.[10,13,23,27,28] Beyond static baseline traits, early on-treatment improvement—typically by Week 12—was the strongest predictor of later low disease activity or remission, underscoring that dynamic response outperforms demographic profiling for prognostication.[11,30] By contrast, age, BMI, and comorbidity rarely predicted response in RCTs,[12–14] although large cohorts consistently implicated current smoking and higher BMI as adverse correlates, with male sex and moderate alcohol intake associated with better outcomes.[15,16,18–20,22,24] These patterns likely reflect effects on inflammatory tone, pharmacokinetics, and adherence rather than MTX-specific pharmacodynamics.

Serological and cellular markers complement clinical assessment by capturing the inflammatory milieu that MTX modulates. Acute-phase reactants (CRP, ESR), MMP-3, and extracellular matrix neo-epitopes (CRP metabolite, C3M, C4M) tracked or preceded clinical change under MTX in RCT or post-hoc analyses,[40,41] and composite panels such as MBDA mirrored disease activity in MTX-based strategies.[42,43] However, conventional autoantibodies had limited predictive value for clinical response—ACPA being primarily prognostic for structural progression rather than predictive of short-term MTX efficacy.[5,27,38,62,63] Mechanistic data converged on adenosine signaling: higher CD39 on T cells and expression of *AMPD1/ADORA2B/ADORA3* predicted remission more strongly than baseline DAS28, suggesting a biologically plausible axis for MTX benefit.[49,50] Additional (immuno)serological signals—including CXCL13, sCD14, regulatory B-cell frequency, and monocyte expansion—showed consistent directions of effect across cohorts, but require standardized cut-offs and external validation.[7,39,45,47,48]

Pharmacogenetic and genetic predictors were heterogeneous. Single-locus effects in folate/purine metabolism and transport (e.g., *MTHFR*, *RFC1/SLC19A1*, *ATIC*, *TYMS*, *MTRR*) were modest and population-dependent.[16,17,61,71,92,93] Although multivariable clinical–pharmacogenetic scores improved discrimination over clinical variables alone,[16,25,75] their incremental value is small relative to the information contained in early clinical response. In contrast, epigenetic classifiers (chromosome conformation; cell-type-resolved DNA methylation) and integrative, machine-learning frameworks achieved stronger performance, likely because they encode both genetic predisposition and contemporaneous disease biology.[54,67,73,74,76,79,89] Still, platform heterogeneity, threshold instability, and limited replication currently preclude routine deployment. Although molecular biomarkers hold promise, clinical and strategic adjustments currently provide the most actionable guidance in practice.

Among all predictors, treatment strategy was the most practical and impactful factor to optimize. Subcutaneous initiation improved short-term responses versus oral administration in head-to-head RCTs and observational cohorts.[80,83] Rapid dose escalation within structured treat-to-target frameworks, sometimes with brief low-dose glucocorticoid bridging, accelerated disease control and reduced radiographic progression without clear safety trade-offs.[11,13,28] Although RCTs support escalation to doses of 20–25 mg/week, real-world practice often remains more cautious, particularly in Asian populations, where safety concerns may favor lower starting or maintenance doses. This should be interpreted within the broader therapeutic window—or window of opportunity—in RA, namely the critical early phase, ideally within 3–6 months of symptom onset, during which prompt DMARD initiation can most effectively suppress inflammation, prevent irreversible joint damage, and improve long-term outcomes. Within this window, the challenge is not only to start treatment early, but also to achieve sufficient exposure while minimizing intolerance. Interindividual variability in intracellular MTX disposition, potentially influenced by transporter genes such as *ABCC1* and *ABCB1*, may partly explain why some patients respond adequately to lower doses, whereas others require more intensive dose escalation. Folate supplementation halved toxicity-driven discontinuations and enabled higher achieved MTX doses.[10,12] Erythrocyte MTX-polyglutamates offered a plausible exposure biomarker for dose individualization and hepatotoxicity surveillance, though influenced by BMI and albumin, warranting prospective algorithms that integrate pharmacokinetics with clinical targets.[46,82] Real-world evidence reinforced the centrality of early, adequate dose intensity and 12-week reassessment; lack of early improvement should trigger timely intensification.[22,30,38,84,85] Patient factors such as high BMI consistently attenuated response and increased combination csDMARD use, arguing for proactive strategy adjustment,[24] potentially reflecting a pharmacokinetic dilution effect whereby increased volume of distribution in obese patients leads to lower effective drug concentrations at standard doses. Similarly, smoking—consistently associated with poorer MTX response—should be actively addressed in clinical practice, with smoking cessation strategies integrated into treatment plans rather than considered an isolated prognostic factor. Sex-related differences, while partly reflecting prescribing patterns,[19] may also have a pharmacokinetic basis, as males typically exhibit greater muscle mass and higher glomerular filtration rates, potentially influencing MTX distribution and tolerance to adverse effects. Importantly, common potential confounders such as caffeine intake did not blunt MTX efficacy.[86]

These findings support a pragmatic, risk-adapted approach to first-line MTX: prompt initiation (ideally within 3 months of symptom onset), consideration of the subcutaneous route, routine folate rescue, rapid dose escalation, and decisive assessment at approximately 12 weeks using composite indices supplemented by parsimonious biomarkers (e.g., CRP and, where available, MMP-3 or MBDA proteins).[10,13,40,42,44] In

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patients with persistent high activity—particularly smokers or those with elevated BMI—early step-up to combination csDMARDs or biologics is warranted.[11,14,22,24] Emerging markers—adenosine-pathway competence, MTX-polyglutamates, cell-specific methylation, and selected soluble mediators (CXCL13, sCD14)—are promising adjuncts for research-guided stratification but should complement, not replace, treat-to-target principles.[7,45,49,50,67,76,82,89]

Limitations of the evidence include heterogeneity in response definitions, dosing, folate schedules, and co-therapies across eras; residual confounding and channeling bias in observational data; small, single-centre biomarker studies with limited external validation; and ancestry-specific genetic effects.[12,21,31,87,88] Future work should randomize actions based on predictors (e.g., MTX-polyglutamate–guided dosing; adenosine-signature–guided monotherapy vs. upfront combination), report calibrated decision thresholds and net benefit, and validate parsimonious, assay-agnostic panels across health systems.[25,73,74]

In sum, early clinical response and modifiable treatment strategy remain the most powerful and immediately actionable predictors of MTX success, while serological, cellular, and multi-omic signatures add biologic plausibility and incremental stratification in selected contexts.[10,11,13,41,50] A disciplined treat-to-target approach that integrates prompt initiation, adequate dosing, early reassessment, and judicious incorporation of emerging biomarkers offers the most credible route to individualized MTX therapy in RA.[40,42,67,84]

An additional strength of this systematic review is its breadth and methodological rigor. A total of 17,250 records were initially identified across PubMed/MEDLINE, Scopus, and CENTRAL databases, and after comprehensive screening and quality assessment, 87 studies were ultimately included. This extensive inclusion process, adhering to PRISMA 2020 guidelines, underscores the robustness and representativeness of the synthesized evidence, covering diverse study designs, populations, and analytic frameworks. Such breadth enhances the generalizability of our conclusions and provides one of the most comprehensive overviews to date on predictors of MTX response in RA.

4.1 | Proposed Risk-Adapted approach for methotrexate initiation in rheumatoid arthritis

Drawing upon the predictors identified in this systematic review, the authors propose a risk-adapted approach to MTX initiation and optimization in RA (Supporting information: Supplementary Material C). This framework integrates established clinical and demographic factors with emerging serological, immunological, and pharmacogenetic markers to support a more individualized therapeutic strategy. Rather than a prescriptive protocol, it represents a structured decision-making process aimed at maximizing early disease control while minimizing unnecessary therapeutic delay.

The approach begins with confirmation of RA according to ACR/EULAR classification criteria and characterization of disease stage. Early disease—defined as symptom duration of less than 3 months—represents the critical "window of opportunity" during which MTX exerts its greatest potential for inducing remission and preventing irreversible joint damage. Baseline assessment should include quantification of disease activity (DAS28, SDAI, or CDAI), evaluation of functional status via the HAQ, and imaging to detect erosive changes.

Subsequent therapeutic decisions are guided by the patient's prognostic and demographic profile. Individuals presenting with low to moderate disease activity, preserved function, and non-erosive disease are generally most likely to respond favorably to MTX monotherapy. Male sex, moderate alcohol intake, and, in some studies, older age, have been associated with better outcomes. Conversely, high baseline inflammatory burden, erosive disease, obesity, and active smoking predict suboptimal response, identifying a subgroup that may warrant early intensification or combination therapy from the outset.

Integration of serological and immunological information refines this risk assessment. Patients with moderate baseline CRP or ESR, early reductions in these markers within 4–12 weeks, low sCD14, elevated CXCL13, or increased $CD39^{+}$ regulatory T-cell frequency—reflecting intact adenosine-mediated immunoregulation—tend to experience superior outcomes on MTX. In contrast, persistently high CRP or ESR, expansion of inflammatory monocyte subsets, or reduced CD39 expression suggest a refractory inflammatory state. Although not yet standardized for routine practice, such biomarkers may complement clinical evaluation in specialized settings or prospective stratification trials.

Pharmacogenetic and pharmacogenomic data provide an additional, albeit limited, layer of precision. Variants in folate and purine metabolism or transport genes—such as *MTHFR*, *RFC1/SLC19A1*, *ATIC*, *TYMS*, and *MTRR*—have been inconsistently linked to MTX efficacy and toxicity. Certain alleles (*MTHFR* 1298C or 677T, *GGH* – 401CC, *RFC1* 80AA) may predispose to adverse events or reduced intracellular drug accumulation, justifying closer monitoring and robust folate supplementation. However, the overall predictive capacity of single-locus genotyping is modest, and routine pre-treatment testing remains unjustified outside research contexts. More promising are multivariable and machine-learning models that combine genetic, biochemical, and clinical features, achieving superior discrimination in external validation cohorts.

Therapeutically, MTX should be initiated promptly once RA is diagnosed, preferably within 3 months of symptom onset. A starting dose of approximately 15 mg/week is appropriate, with the subcutaneous route favored in patients with obesity, gastrointestinal intolerance, or high inflammatory activity. Rapid escalation to 20 mg/week or 0.3 mg/kg by 6 to 8 weeks enhances efficacy when tolerated. Folic or folinic acid supplementation should be routine to reduce toxicity,

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and short-term low-dose prednisone (≤10 mg/day for 6–12 weeks) may accelerate early disease control in treat-to-target strategies.

The first decisive reassessment should occur at approximately 12 weeks. Early suppression of disease activity—reflected by at least 20%–30% improvement in composite indices or achievement of a EULAR moderate/good response—is the most reliable predictor of long-term remission. Patients demonstrating early improvement should continue optimized MTX monotherapy, while those with minimal or absent response—particularly if associated with high BMI, smoking, or unfavorable biomarker profiles—should be considered for therapeutic escalation. In partial responders with good tolerance, switching from oral to subcutaneous administration or further dose intensification may restore efficacy. Persistent disease activity beyond 24 weeks, despite optimal dosing and adherence, warrants combination csDMARD therapy (e.g., MTX with sulfasalazine or leflunomide) or transition to a biologic or targeted synthetic DMARD, depending on comorbidities, access, and treatment goals.

Throughout therapy, regular monitoring remains essential. Laboratory surveillance for hematologic and hepatic toxicity should accompany serial assessment of disease activity every 8–12 weeks until remission or low disease activity is achieved. Optional tools such as erythrocyte MTX-polyglutamate levels, MBDA scores, or power Doppler ultrasonography may refine dosing decisions and detect subclinical inflammation. In patients with sustained remission, cautious MTX dose tapering can be considered, though relapse risk remains higher in those with erosive disease or persistent autoantibody positivity.

The proposed algorithm integrates multiple sources of evidence into a clinical pathway emphasizing early treatment initiation, dynamic reassessment, and timely therapy adjustment. Early suppression of inflammation emerges as the key determinant of treatment success, surpassing static demographic or genetic factors. While biological and pharmacogenetic data may enhance precision, they should complement—rather than replace—clinical judgment and treat-to-target principles. Implementation remains limited by heterogeneity across studies, small sample sizes, and the lack of standardized or externally validated biomarkers. These findings may inform the design of stratified randomized controlled trials and contribute to the development or refinement of RA treatment guidelines. Consequently, this framework should be regarded as an evolving model, grounded in robust evidence supporting early and optimized MTX use, while remaining adaptable to emerging biomarkers and predictive tools.

5 | CONCLUSIONS

This systematic review integrated a broad and heterogeneous body of evidence to identify the most reliable predictors of MTX response in RA. This synthesis was essential to distinguish consistent predictors—early disease suppression, timely MTX initiation, subcutaneous administration, and folate supplementation—from findings with limited or conflicting support. It also revealed persistent gaps, particularly regarding the reproducibility of genetic and multi-omic markers, which, despite promising accuracy in research settings, remain largely inaccessible and should currently be regarded as investigational tools rather than routine clinical tests.

Overall, this review highlights that early and optimized treatment strategies constitute the most effective, and evidence-based approach, and that current clinical decision-making should prioritize readily available indicators (e.g., CRP, DAS28), complemented where appropriate by a limited set of validated biomarkers (e.g., MBDA, MMP-3), to ensure practical applicability without overburdening clinicians, while ongoing research into validated biological predictors is essential to enable truly individualized MTX therapy in RA.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception and design of the study, data acquisition, analysis, and interpretation. All authors were involved in drafting the manuscript and revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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The authors have nothing to report.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

ETHICS STATEMENT

This study is a systematic review of the literature. It did not involve the collection of primary data and did not include human participants or animal subjects. Therefore, ethical approval and informed consent were not required.

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GRAPHICAL ABSTRACT

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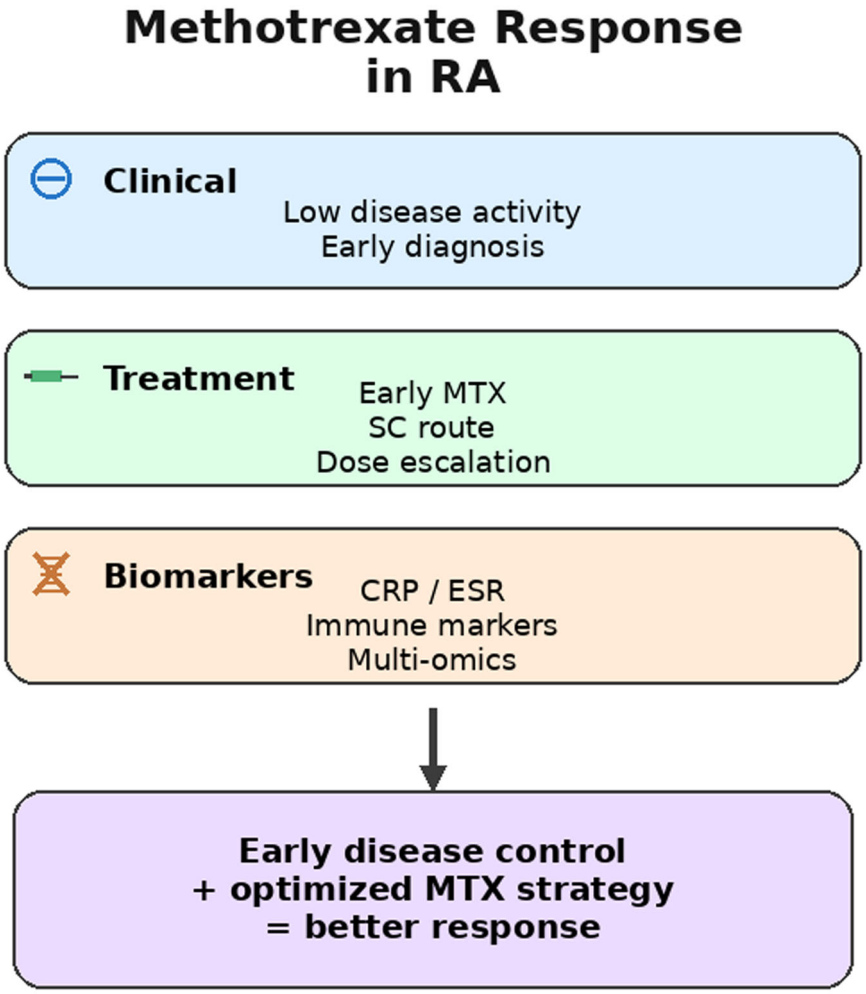

This review integrates clinical, immunological, genetic, and multi-omic evidence, highlighting dynamic inflammatory and adenosine-pathway biomarkers and reinforcing treat-to-target principles as the most actionable framework for personalized methotrexate therapy.

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