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META-ANALYSIS

A meta-analysis of methotrexate polyglutamates in relation to efficacy and toxicity of methotrexate in inflammatory arthritis, colitis and dermatitis

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Aims: In immune-mediated inflammatory diseases (IMIDs), early symptom control is a key therapeutic goal. Methotrexate (MTX) is the first-line treatment across IMIDs. However, MTX is underutilized and suboptimally dosed, partly due to the inability of making individualized treatment decisions through therapeutic drug monitoring (TDM). To implement TDM in clinical practice, establishing a relationship between drug concentration and disease activity is paramount. In this meta-analysis, we investigated the relationship between concentrations of MTX polyglutamates (MTX-PG) in erythrocytes and efficacy as well as toxicity across IMIDs.

Methods: Studies analysing MTX-PG in relation to disease activity and/or toxicity were included for inflammatory arthritis (rheumatoid [RA] and juvenile idiopathic arthritis [JIA]), inflammatory bowel disease (Crohn's and ulcerative colitis) and dermatitis (psoriasis and atopic dermatitis). Meta-analyses were performed resulting in several summary effect measures: regression coefficient (β), correlation coefficient and mean difference (of MTX-PG in responders *vs.* nonresponders) for IMIDs separately and collectively.

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Results: Twenty-five studies were included. In RA and JIA, higher MTX-PG was significantly associated with lower disease activity at 3 months (β: −0.002; 95% confidence interval [CI]: −0.004 to −0.001) and after 4 months of MTX use (β: −0.003; 95% CI: −0.005 to −0.002). Similarly, higher MTX-PG correlated with lower disease activity in psoriasis (R: −0.82; 95% CI: −0.976 to −0.102). Higher MTX-PG was observed in RA, JIA and psoriasis responders (mean difference: 5.2 nmol/L MTX-PG_{total}; $P < .01$).

Conclusion: We showed that higher concentrations of erythrocyte MTX-PG were associated with lower disease activity in RA, JIA and psoriasis. These findings are an important step towards implementation of TDM for MTX treatment across IMIDs.

KEYWORDS
immune-mediated inflammatory diseases, methotrexate, pharmacodynamics, therapeutic drug monitoring

1 | INTRODUCTION

Immune-mediated inflammatory diseases (IMIDs), such as inflammatory arthritis, inflammatory bowel diseases (IBD) and dermatitis, have emerged as a public health challenge, affecting >20 million people worldwide (estimated prevalence of 0.2–0.56%) with the highest prevalence in Europe and North America.[1–3] These diseases result in substantial reductions in patients' quality of life and work productivity and considerable health care costs, due to the necessity of expensive therapeutic interventions. Consequently, the successful treatment of these diseases has become a key health priority.

In inflammatory arthritis (rheumatoid arthritis [RA], psoriatic arthritis [PsA] or juvenile idiopathic arthritis [JIA]), IBD (Crohn's disease [CD], ulcerative colitis [UC] or unclassified [IBD-U]) and dermatitis (atopic dermatitis [AD] or psoriasis [Ps]), early achievement of effective symptom control is a key therapeutic goal. In RA and JIA, the first drug of choice is methotrexate (MTX) because of its efficacy and long-term safety.[4,5] In moderate to severe psoriasis and severe atopic dermatitis, MTX is also a first-line treatment option, albeit not Food and Drug Administration/European Medicines Agency-approved for use in atopic dermatitis.[6,7] In CD, MTX is recommended for mild disease either as a first-line steroid-sparing agent or as second-line therapy in patients who failed to respond to thiopurines.[8] Even in RA, where MTX is considered an anchor first-line disease-modifying drug due to low costs, efficacy and extensive clinical experience, MTX is underutilized and suboptimally dosed in 65% of patients.[9] One of the reasons for suboptimal use may be the inability to make individualized treatment decisions, for example, through therapeutic drug monitoring (TDM), which comprises determination of drug concentrations in the individual patient with subsequent dose adaptation to improve efficacy, minimize toxicity and/or assess drug adherence. To apply TDM, drug concentrations should be quickly and reliably measured in an accessible body compartment, interindividual variation should exist and a relationship between the drug concentration and clinical effect needs to be established.[10]

TDM is currently not utilized for MTX in IMIDs, mostly due to MTX's complex metabolism. Plasma MTX cannot be used as a biomarker for efficacy since MTX is transported intracellularly within 24 h by transporters such as the reduced folate carrier leaving no stable and measurable plasma concentrations. Intracellularly, the enzyme folylpolyglutamate synthetase subsequently adds up to 7 glutamate residues to MTX (MTX-PG_{1-7}). Polyglutamylation results in cellular retention and inhibition of the target enzymes in the folate and adenosine pathway. MTX-PG concentrations can be measured by mass spectrometry techniques in, amongst others, erythrocytes.[11] Our group showed in 2 prospective studies that higher red blood cell (RBC) MTX-PG_{3-5} in RA[12] and JIA[13] were associated with lower disease activity and that considerable interindividual variation in the reached concentrations existed. This suggests that measurements of RBC MTX-PG may provide a valuable TDM tool for the clinician to individualize MTX dosing in an early phase of treatment, to achieve faster disease remission and less (end-organ) damage and/or side effects. However, the available data on the relationship between RBC MTX-PG concentrations and clinical efficacy or toxicity in individual IMIDs are still conflicting.[14,15]

In this meta-analysis, we investigated the relationship between concentrations of erythrocyte MTX-PG (species) and therapeutic efficacy across IMIDs as a primary research question, as well as the relationship between concentrations and toxicity as a secondary research question.

2 | METHODS

2.1 | Search strategy and study selection

Studies involving patients with inflammatory arthritis (RA, PsA and JIA), colitis (CD, UC and IBD-U) and dermatitis (AD and Ps) on MTX, which analysed erythrocyte MTX-PG in relation to disease activity

and/or toxicity, were included. Other (off-label) indications were not included. We only analysed studies reporting erythrocyte MTX-PGs. Inclusion and exclusion criteria are provided in Table 1. A comprehensive literature search using MEDLINE (from onset in 1946) and Embase (from onset in 1974) was conducted on 14 November 2019. On 14 January 2022, a new search was conducted yielding 226 new studies. Two RA studies that possibly satisfied our inclusion criteria were critically reviewed; however, they could not be included due to missing correlations between MTX-PG and disease activity. Keywords used in the literature search included a combination of free-text terms in title/abstract and medical subject heading (MeSH) terms of "methotrexate" AND "pharmacokinetics" OR "pharmacodynamics" OR "polyglutamates" AND disease entities, listed in Table 1. The detailed search strategy is provided in File S1. Reference lists of included studies and previous systematic reviews on this topic were screened for additional papers. Also, a forward citation search of our included landmark articles was performed using Scopus (Elsevier, 2021).

The identified studies, titles and abstracts were screened independently, using Covidence (systematic review software, Veritas Health Innovation, Australia), by 2 researchers (M.M. and M.B.) to exclude studies not fulfilling the inclusion criteria for our research question. Discrepancies were resolved through a consensus discussion with the third researcher (R.H.). Next, all reports were assessed thoroughly based on inclusion and exclusion criteria. This process was repeated independently by 2 researchers (M.M. and R.H.) using Covidence with a third researcher (M.B.) available to resolve discrepancies. Multiple reports on the same study were included if the second report published additional relevant information. We used the PRISMA guideline for reporting systematic reviews as appropriate.

2.2 | Data collection and quality assessment

The following data of each study were collected: study characteristics (design, inclusion criteria, year of inclusion and publication and number of patients), patient characteristics (disease, duration of disease, age, sex and country), drug characteristics (folic acid use, MTX dose and route of administration, concomitant drugs, and method and timing of MTX-PG quantification), parameters of efficacy and toxicity (disease activity score, definition of remission and definition of toxicity), effect sizes and statistics (*P* value, correlation and regression coefficient and odds ratio), and MTX-$PG_{1-5/total}$ concentrations (determined in cross-sectional studies or longitudinally between 1 and 12 months after MTX start, separate species, combined and total). Authors were contacted if the published data were insufficient to answer our research questions. In case of multiple reports of 1 study, the primary and the secondary studies were selected, and information was collected separately. Data from derivation and validation cohorts were also collected separately.

Medians and ranges were converted to means and standard deviations (SDs) using the method described by Wan et al.[16] Similarly, standard errors of the mean MTX-PG concentration were converted into SDs.[17] Confidence intervals of the β regression coefficient were converted into standard errors.[18] Plot Digitizer was used to extract data from figures essential for answering our primary research question.[19] Published erythrocyte MTX concentrations were considered equivalent to MTX-PG_{total} concentrations. MTX-PG concentrations expressed in nmol/8×10^{12} RBC were considered equivalent to nmol/L as those studies used packed or washed erythrocytes with an RBC SI reference range of 8×10^{12} RBC/L (based on the conversion of the haematocrit value). Any difference between MTX-PG

TABLE 1 Eligibility criteria of selected studies

	Inclusion criteria	Exclusion criteria
Population: *Disease*	Atopic dermatitis (AD), psoriasis (Ps), Crohn's disease (CD), ulcerative colitis (UC), inflammatory bowel disease unclassified (IBD-U), rheumatoid arthritis (RA), psoriatic arthritis (PsA), juvenile idiopathic arthritis (JIA)	Malignancy (leukaemia) or animals
Population: *Medication*	Subcutaneous (sc), intramuscular (im) or oral (po) MTX use	Topical or intra-articular MTX Biologics coadministration (ts/bDMARDS)
Measurement	RBC MTX-$PG_{total/1-7}$ regardless of method of measurement	MTX-PG measured in plasma, white blood cells or tissue. nmol/g Hb or nmol/mmol Hb as a unit of MTX-PG
Outcome	Clinical response expressed in a standardized disease activity score; or Biochemical response CRP, ESR, Faecal calprotectin; or Toxicity of MTX: gastrointestinal, hematologic, hepatic or neurological adverse events	Quality of life as a sole outcome TJC or SJC for 22 joints only
Study design	Case–control, cohort, cross-sectional studies, randomized controlled trials, (Conference) abstracts.	Case report or series, systematic review, population pharmacokinetic–pharmacodynamic models
Other characteristics	Quantitative data applicable	Language in characters.

Abbreviations: bDMARD, biological disease modifying anti rheumatic drug; CRP, c- reactive protein; ESR, eryhtocyte sedimentation rate; MTX-PG, methotrexate polyglutamate; RBC, red blood cell; tsDMARD, targeted synthetic disease modifying anti rheumatic drug.

concentration expressed in nmol/L *vs.* nmol/8 × 10^{12} RBC is likely to be <10%. Therefore, MTX-PG concentrations in nmol/8 × 10^{12} RBC were considered comparable with MTX-PG concentrations in nmol/L.

Two researchers (M.M. and R.H.) extracted data to Castor EDC (Castor Electronic Data Capture, The Netherlands) and mutually reviewed extraction forms on accuracy. The third researcher (M.B.) was available to resolve conflicts. The quality of included full-text studies, with the exception of short communications, was assessed independently by 2 researchers (M.M. and R.H) using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies from the NIH.[20] Disagreements were resolved through a consensus discussion together with the third researcher (M.B.).

2.3 | Data synthesis

Multiple reports of 1 study were synthesized and analysed together as 1 record. All definitions of response were considered equivalent irrespective of the disease activity measure used to define response. Based on the available literature we made an assumption that, by pooling the established disease activity measures, the misclassification of responders as nonresponders or vice versa would be negligible.[21–23] Physician Global Assessment was also considered equal to established disease activity measures and was therefore also pooled with other disease activity measures such as the DAS-28. If a study reported > 1 disease activity score, that with the most complete data and/or the most compatible with other studies was chosen. For example, if both European League Against Rheumatism response criteria and DAS-28 were used to define response, only the DAS-28 response was chosen.

Effect measures, used to describe the relationship of MTX-PG concentration with disease activity/response and/or toxicity, were the β regression coefficient, correlation coefficient, odds ratio and mean difference (MD; of MTX-PG concentrations). Spearman correlation coefficients were transformed to Pearson correlation coefficients to calculate a summary correlation coefficient.[24] The MD and 95% confidence interval (CI) of MTX-PG concentration between responders *vs.* nonresponders as well as between those having toxicity *vs.* no toxicity were calculated from MTX-$PG_{total/1-5}$ concentrations (mean ± SD).

Summary effect measures were calculated for the regression coefficients (pooled either if they were all standardized or all unstandardized), correlation coefficients and the MD of MTX-PG concentration. They were obtained for each IMID as well as for all IMIDs together (deviation from the submitted protocol) if at least 3 studies were available. Furthermore, summary effect measures were shown separately for the time period of 0–3 months *vs.* 4 months and onwards after MTX start, under the assumption that most MTX-PG species reach steady state at around 3 months upon MTX initiation.[25] If a study reported data on multiple time points before or after 3 months, a weighted effect measure of all time points in that period was calculated based on the sample size of that particular time point (if applicable). Cross-sectional studies were included in the analysis for the time period of using MTX 4 months and onwards, as studies (except from Angelis-Stofordis et al.) used inclusion criteria of a minimal use of (a stable dose) MTX for 12 weeks.[26]

2.4 | Data analysis

A random-effects model was applied to obtain summary effect measures, as considerable heterogeneity was expected. Between-study variance in the random-effects model was estimated by a restricted maximum-likelihood estimator.[27] The between-study heterogeneity was quantified with the inconsistency index (I^2) statistic and tested for significance using Cochran's *Q* test. Because this test is underpowered to detect moderate degrees of heterogeneity, *P* values <.10 indicated the presence of heterogeneity.[28]

Several considerations with respect to effect measures are noteworthy. Direction of a correlation coefficient was changed from positive to negative if a positive correlation between high drug levels, and the Δ disease activity score (difference in disease activity at 3 months compared to baseline) was reported. This is because the *actual* disease activity decreases as a result of increased MTX-PG concentration. For example, Chladek et al. reported a ρ, converted to Pearson correlation coefficient of −0.374.[29] A negative correlation therefore signifies that a higher MTX-PG concentrations relates to a lower disease activity. Furthermore, the Pearson correlation coefficient was transformed to a Fisher's *r*-to-*z* transformed correlation coefficient during analysis.[30] The confidence intervals for the effect measures were than calculated using the Fisher to *z* transformation and a large sample approximation (normal distribution) and eventually back transformed to the correlation scale.

Publication bias was assessed visually by a funnel plot if >10 studies were analysed. To perform statistical analyses and construct graphs and plots, R version 4.0.3 (Metafor package) statistical software (The R Foundation for Statistical Computing, Vienna, Austria) was employed.[27] For all tests (with the exception of heterogeneity), *P* values of <.05 were considered statistically significant.

3 | RESULTS

3.1 | Search

Of 4265 studies, 29 were assessed as eligible, which included 25 unique studies and 4 secondary reports of the same study. One study contained data from a derivation and a validation cohort, and these were collected separately, with ultimately 26 cohorts available for analysis.[12] Of these records, 4 comprised abstracts only and 1 was a short communication.[31–36] The flow diagram of study selection is illustrated in Figure S1. Agreement between researchers with respect to study eligibility assessment was good for both title/abstract and full-text screening (κ-statistic 0.69 and 0.76, respectively).

3.2 | Study characteristics

Studies on inflammatory arthritis prevailed. For RA, 8 prospective ($n = 727$) and 6 cross-sectional design ($n = 941$) studies were included while 1 cohort ($n = 113$) and 2 cross-sectional studies ($n = 115$) were included for JIA. Studies on psoriatic arthritis were lacking. For IBD (ulcerative colitis and Crohn's disease), 1 cohort study ($n = 30$) and 2 cross-sectional studies ($n = 30$) were included. For inflammatory skin diseases, psoriasis and atopic dermatitis, 6 cohort studies were included ($n = 188$). It is noteworthy that only 1 study by Rahman et al. included 30 atopic dermatitis patients (combined with psoriasis). Important characteristics of all studies are summarized in Table 2.

Six studies analysed the relationship between drug concentrations and toxicity with data published, whereas 5 studies made only descriptive remarks about the concentration–toxicity relationship.

We requested authors of several studies to provide raw data and received them from Woolf et al.,[37] Chladek et al.,[38] Bulatović Ćalasan et al.[13] and de Rotte et al.[12]

Patients were enrolled from 1995 to 2015. The majority was female, particularly in RA (74%) and JIA (68%). Mean weighted age of patients was 57.4 years in RA, 11.3 years in JIA, 48.7 years in IBD and 37.9 years in Ps/AD. Mean duration of disease was 35.2 months for RA patients (data from 9 studies available), 58.8 months (1 study) for JIA, 92.5 months (2 studies) for IBD and 258.7 months for Ps/AD patients (2 studies). Mean duration of MTX use was 42.9 months in RA (8 studies), 47.1 months in JIA (2 studies), 12.1 months in IBD (3 studies) and 2.9 months in Ps/AD (6 studies). Adherence was reported in only 3 studies.[12,39,40]

The vast majority (90.4%) of RA patients used oral MTX (data from 9 studies available), in contrast to JIA patients (68.9%) and IBD patients (10.0%). MTX dosing in Japan was lower compared with other countries.[41,42] In cross-sectional studies, patients were only included if they were on (stable) MTX dosage for at least 3 months.

The MTX-PG measurement method, described by Dervieux et al., based on high-performance liquid chromatography (LC), was used in 11 studies.[43] MTX-PG concentrations were also measured by enzyme ligand assays ($n = 4$) and competitive protein binding assay ($n = 1$). Using these techniques, only MTX-PG_{total} rather than individual MTX-PG_{1-7} species can be quantified. More recent studies ($n = 5$) used ultra-performance LC–tandem mass spectrometry (MS/MS), of which 2 studies used a stable-isotope-labelled internal standard.[12,13] Three studies reported drug levels in nmol/8 $\times$ 10^{12} RBCs.[44–46]

3.3 | Quality assessment

The overall quality of the full-text studies (with the exception of short communications) was graded as good in 4 studies, fair in 11 studies and as poor in 5 studies (Table S1). The RA studies were either fair (6/9) or good (3/9). One JIA study was good, and 1 study was assessed as poor. In IBD, 1 study was graded as fair and the remaining 2 as poor. Ps/AD studies were predominantly fair (4/5).

3.4 | Overall MTX-PG concentrations

Figure 1A shows the reported MTX-PG_{total} levels independent of disease activity and/or toxicity. MTX-PG_{total} concentrations do not differ between diseases or between measurement time-points. However, the range is wide (25–825 nmol/L) showing large interpatient variability. Also, the measurement method does not appear to influence the MTX-PG_{total} levels (Figure 1B). Importantly, Figure 1B does not reflect the precision of the lab method at hand. Fischer et al., considered as a study of poor quality, reported aberrantly high drug levels compared with other studies and methods.[46] Figure 2 shows the proportion of MTX-PG species in studies that reported levels independently of disease activity and/or toxicity. MTX-PG_1 is the predominant species in the first 3 months whereas MTX-PG_3 is the most predominant species after 4 months of MTX use. This is in line with multiple studies that reported MTX-PG_3 as the most prevalent subtype, comprising 38–41% of MTX-PG_{total}.[34,36,39] Furthermore, Figure 2 demonstrates that MTX-PG_{3-5} concentrations increase over time at the expense of MTX-PG_{1-2}.

3.5 | Relationship between disease activity and MTX-PG concentration

3.5.1 | Regression coefficient

Three studies and 4 cohorts ($n = 495$) employed β regression coefficients as the effect size to describe the relationship between disease activity and the MTX-PG_{total} concentration in the first 3 months after MTX start.[12,13,41] (Figure 3A) Although the summary β was not statistically significant for RA studies only ($n = 385$; due to the wide confidence interval of Takahashi et al.[41]), a significant summary β of −0.002 was found for RA and JIA studies combined (Figure 3A). Of note is that the large confidence interval of Takahashi et al. is due to summation and weighing of means and SEs of MTX-PG concentration at various time-points in this study. After 4 months of MTX use, the summary β's of RA studies only ($n = 326$; 4 studies) and RA and JIA studies together ($n = 427$; 5 studies) were both significant (−0.004 and −0.003, respectively; Figure 3B). There were no subgroup differences, and heterogeneity might therefore not be important. All studies were of good quality. Taken together, these results signify that higher MTX-PG concentrations are associated with lower disease activity.

The forest plots of MTX-PG_{1-5} species are shown for months 1–3 after MTX start (Figure 4A) and after 4 months of MTX use (Figure 4B). The summary βs of MTX-PG_2, MTX-PG_3 and MTX-PG_4 were significant with a comparable trend to the MTX-PG_{total} concentration.[12,13,41,46,47] Studies with IBD and Ps/AD patients did not report any βs of the MTX-$PG_{1-5/total}$ concentration in relation to disease activity.

TABLE 2 Study characteristics

Study	Population					Methotrexate			
	Country	N	Age (y)[a]	F (%)	DA[b,a]	Oral (%)	Dose (mg/wk)[a]	Duration use (m)[a]	Co-therapy (%)
Rheumatoid arthritis – RCT/cohort studies									
De Rotte, 2015. MTX-R cohort	NL	102	52 ± 16	71	4.3 ± 1.4	94	15 ± 2	0	Folic acid (100) Steroids (14) NSAID (36) cDMARD (57)
De Rotte, 2015. tREACH cohort	NL	285	54 ± 14	70	4.9 ± 1.2	100	25 ± 1	0	Folic acid (100) Steroids (94) NSAID (14) cDMARD (62)
Dervieux, 2006	USA	48	55 ± 15	n/a	5.1 ± 1.1	100	14 ± 2	0	Folic acid (96) Steroids (58) NSAID (63) cDMARD (29)
Hobl, 2012	Austria	19	56 ± n/a	68	4.7 ± n/a	100	Min 15 – Max 25	0	Folic acid (100) Steroids (n/a) NSAID (n/a)
Murosaki, 2017	Japan	42	64 ± 12	71	5.3 ± 1.1	100	5 ± 1	0	Folic acid (47) Steroids (40) NSAIDs (72) cDMARD (46)
Nishida, 2018 [A]	Japan	35	n/a	n/a	n/a	n/a	n/a	0	n/a
Sandhu, 2017[b] and Sandhu, 2014 [A]	India	117	43 ± 12	88	6.3 ± 0.8	76	22 ± 4	0	Folic acid (100) Steroids (27) cDMARD (4)
Takahashi 2017[b] and Takahashi 2014 [A]	Japan	79	57 ± 15	86	4.0 ± 1.1	n/a	13 (8–16)	0	Folic acid (100) Steroids (8) NSAIDs (34) cDMARD (9)
Rheumatoid arthritis – Cross-sectional studies									
Angelis-Stoforidis, 1999	Australia	99	57 ± 11	62	n/a	98	(2.5–37.5)	>2	Folic acid (5) Steroids (54)

TABLE 2 (Continued)

Study	Population					Methotrexate			
	Country	N	Age (y)[a]	F (%)	DA[b,a]	Oral (%)	Dose (mg/wk)[a]	Duration use (m)[a]	Co-therapy (%)
									NSAIDs (35) cDMARD (60)
Dervieux, 2009 [A][b] and Dervieux 2009 [A]	USA	256	n/a	n/a	n/a	n/a	15 ± 4	>3	n/a
Dervieux, 2004	USA	108	65 ± 11	77	n/a	n/a	15 ± 4	100 ± 52	Folic acid (84) Steroids (49)
Dervieux, 2005	USA	226	66 ± 13	73	n/a	n/a	14 ± 6	56 ± 58	Folic acid (81) Steroids (47)
Gridneva, 2019 [A]	Russia	60	n/a	73	n/a	0	Min 20	>3	Steroids (43)
Stamp, 2010	NZ	192	61 ± n/a	73	n/a	100	15 ± 4	76 ± 41	Folic acid (99) Steroids (31) NSAIDs (45) cDMARD (10)
Juvenile idiopathic arthritis – Cohort studies									
Bulatovic Calasan 2015	NL	113	11 ± 5	68	12.7	98	14 ± 2	0	Folic acid (100) NSAID (81)
Juvenile idiopathic arthritis – Cross sectional studies									
Becker 2009 [A]	USA	85	10 ± 5	72	n/a	36	0.5 ± 0.3 mg/kg	49 ± 31	n/a
Dolezalova 2005	Czech Republic	30	12 ± 4	60	1.3 ± 2	53	14 ± 5	41 ± 28	Folic acid (100) Steroids (13) NSAID (100) cDMARD (17)
Inflammatory bowel disease – Cohort studies									
Egan, 1999	USA	30	45 ± 13	45	138 ± 38	0	15 or 25	0	Steroids (100) 5-ASA (63)
Inflammatory bowel disease – Cross sectional studies									
Brooks, 2007	NZ	18	51 ± 15	50	124 ± 109	22	20 ± 2.7	26 ± 15	Steroids (39) 5-ASA (78)
Fischer, 2017 and Shivi, 2014 [A]	USA	12	54 ± 12	33	5 ± 3.5	17	25 ± 0	21 ± 12	Folic acid (100) Steroids (25)
Psoriasis and atopic dermatitis – Cohort studies									
Woolf 2012	UK	55	44 ± 12	29	12 ± 6	100	15 ± 13	0	Folic acid (n/a)

TABLE 2 (Continued)

Study	Population					Methotrexate			
	Country	N	Age (y)[a]	F (%)	DA[b,a]	Oral (%)	Dose (mg/wk)[a]	Duration use (m)[a]	Co-therapy (%)
Hroch 2008	Czech Republic	16	53	75	24 ± 8	100	14 (7.5–22.5)	0	n/a
Chladek 1998	Czech republic	10	48 ± 17	70	26 ± 19	100	15	0	n/a
Chladek 2005	Czech Republic	41	46 ± 12	41	30 ± 17	n/a	(7.5–15)	0	n/a
Chladek 2008	Czech republic	20	50	50	24 ± 8	100	14.6	0	Folic acid (n/a)
Rahman 2014	USA	46	9 ± 13	n/a	n/a	n/a	(n/a–15 mg)	≥12	n/a

Abbreviations: [A], abstract only; BM, bone marrow; CDAI, Crohn's disease activity index; cDMARD, conventional disease-modifying anti-rheumatic drugs; CNS, central nervous system; CRP, C-reactive protein; CZ, Czech Republic; DAS-28, Disease Activity Score of 28 joints; ESR, erythrocyte sedimentation rate; EULAR, DAS-based European League against Rheumatism response criteria; F, female; GI, gastrointestinal; IBDQ, Inflammatory Bowel Disease Questionnaire; m, months; mg/wk, milligram per week; *N*, number; n/a, not applicable; NL, The Netherlands; NSAIDs, nonsteroidal anti-inflammatory drugs; NZ, New Zealand; PGA, physician global assessment; RCT, randomized controlled trial; SJC, swollen joint count; TJC, tender joint count; wk, week; y, years; 5-ASA, 5-aminosalicyclic acid containing drugs.

[a]Mean ± standard deviation (unless not given), (range).

[b]Two articles of the same study. Mark is set by primary study.

[c]Time point of MTX-PG measurement is used for the analysis of the relationship with disease activity.

[d]Result of disease activity score at baseline for cohort studies and at the moment of analysis for cross-sectional studies. Scores used: for RA DAS-28; for JIA JADAS; for IBD the disease activity index.

Definitions of response:

[1]Delta used for (regression) analysis.

[2]Moderate or good EULAR response.

[3]Delta DAS-28 is greater than the median of 41%.

[4]Any improvement.

[5]Delta DAS-28 > 1.2.

[6]EULAR good response.

[7]Classified by (same treating) rheumatologist based on physician's global clinical assessment which included the patient history, joint examination and a comparison with the patient's condition at an earlier point in time, that is, a comparative temporal assessment.

[8]Physician global assessment of response scored by visual analogue scale (0 = high response, 10 = poor response). Responders had score of 2 cm or less.

[9]DAS-28 ≤ 3.2.

[10]IBDQ score ≥170 and discontinuation of prednisone.

[11]CDAI ≤ 150.

[12]Decrease in HBI of ≥3.

[13]PASI 50% improvement.

[14]Percentage of the initial PASI score.

[15]PASI 75% improvement.

[16]Three core sets improved by >30% and not more than 1 worsened by 30% (Reference: Gianninni 1997). Core sets: overall well-being, functional ability, no. of active joints, no. of joints with limited range of motion.

Definition of toxicity:

[a]Diarrhoea, nausea, vomiting, abdominal pain, mouth ulcers, decreased appetite, dyspepsia and/or stomatitis.

[b]Alanine aminotransferase 2–3 times upper level of normal (or above).

[c]Fatigue, dizziness, headache, sleeplessness, not feeling well, psychological complaints, anxiety, lethargy, loss of concentration, blurred vision, sleep disturbance, weepiness and/or forgetfulness.

[d]Leucocytes < 3×10^9/L and/or thrombocytes < 100×10^9/L.

[e]Elevation of aspartate aminotransferase above the upper limit of normal.

[f]Lymphocyte count < 0.9×10^9/L, granulocyte count < 1.5×10^9/L and/or thrombocyte count < 20×10^9/L.

[g]White blood cell count < 3.5×10^9/L, haemoglobin < 8 g/L, mean corpuscular volume > 120 fL.

[h]Elevation of aspartate aminotransferase or alanine aminotransferase above the upper limit of normal.

[i]Leucocytes < 4×10^9/L and/or thrombocytes < 150×10^9/L.

[j]Abnormalities of liver function necessitating dose reduction.

[k]Any difference in the complete blood cell count.

[l]Elevation of alkaline phosphatase, aspartate aminotransferase or alanine aminotransferase above the upper limit of normal.

[m]Alanine aminotransferase above upper level of normal and serum procollagen 3 above 4.2 μg/L.

[n]White blood cell < 4×10^9/L and/or neutrophil count < 1.5×10^9/L.

TABLE 2 (Continued)

Study	MTX-PG measurement			Outcome	
	Timing (wk)	Method	PG species	Disease activity score	Toxicity
Rheumatoid arthritis – RCT/cohort studies					
De Rotte, 2015. MTX-R cohort	12[c], 24[c], 36[c]	UPLC-MS/MS with internal standard	1–5	DAS-28[1] EULAR[2]	GI[a], Liver[b], CNS[c], BM[d]
De Rotte, 2015. tREACH cohort	12[c], 24[c], 36[c]	UPLC-MS/MS with internal standard	1–5	DAS-28[1]	GI[a], Liver[b], CNS[c], BM[d]
Dervieux, 2006	4, 8, 12, 16[c], 20, 24[c]	HPLC	Total	DAS-28[1,3] EULAR[2] PGA[1]	GI[a], Liver[e], CNS[c]
Hobl, 2012	1, 5[c], 10[c]	HPLC	1-5	DAS-28-ESR[1,4] (week 16), CRP[1], ESR[1]	n/a
Murosaki, 2017	2, 4, 8, 12, 24[c]	HPLC	1–5	DAS-28[5]	n/a
Nishida, 2018 [A]	4[c], 12[c], 24[c], 48	LC–MS/MS	1–4	DAS-28[1] EULAR[6]	n/a
Sandhu, 2017[b] and Sandhu, 2014 [A]	4[c], 8[c], 16[c], 24[c]	HPLC	3, 3–5, total	EULAR[2] DAS-28-3	GI[a], Liver[h], CNS[c], BM[l]
Takahashi 2017[b] and Takahashi 2014 [A]	4[c], 8[c], 12[c], 24[c], 36[c], 52[c], 76[c]	HPLC	1–3, total	DAS-28[1,5] EULAR[6]	Liver[h]
Rheumatoid arthritis – Cross-sectional studies					
Angelis-Stoforidis, 1999	n/a	Enzyme ligand assay	Total	PGA[7]	GI[a], Liver[j]
Dervieux, 2009 [A][b] and Dervieux 2009 [A]	n/a	HPLC	1–5	EULAR[6] or PGA[8]	n/a
Dervieux, 2004	n/a	HPLC	3	PGA[8]	n/a
Dervieux, 2005	n/a	HPLC	3	PGA[8] TJC[1]	n/a
Gridneva, 2019 [A]	n/a	HPLC	1–5, total	EULAR[2]	n/a
Stamp, 2010	n/a	HPLC	1–5, total	DAS-28[9] PGA, TJC etc. (no definitions)	GI[a], Liver[e], CNS[c], BM[k]
Juvenile idiopathic arthritis – Cohort studies					
Bulatovic Calasan 2015	12[c]	UPLC-MS/MS with internal standard	1–5	JADAS-27[1]	GI[a], Liver[b], BM[f]
Juvenile idiopathic arthritis – Cross sectional studies					
Becker 2009 [A]	n/a	Ion pair UPLC-ESI-MS/MS	1–5	Any active arthritis	n/a
Dolezalova 2005	n/a	Enzyme ligand assay	Total	Giannini[16]	GI[a]
Inflammatory bowel disease – Cohort studies					
Egan, 1999	6, 8, 10, 12, 14, 16 ([c]mean of 6–16)	Competitive protein binding assay	Total	IBDQ[10]	n/a
Inflammatory bowel disease – Cross sectional studies					
Brooks, 2007	3 times in 1 month ([c]mean of 3)	HPLC	1–5	CDAI[11]	GI[a], CNS[c]
Fischer, 2017 and Shivi, 2014 [A]	n/a	LCMS without internal standard	1–5	HBI[12]	GI[a], Liver[l], CNS[c]
Psoriasis and atopic dermatitis – Cohort studies					
Woolf 2012	4, 8, 12, 24[c], 52	HPLC	1–5	PASI[13]	GI[a], Liver[m], BM[n]
Hroch 2008	4, 8, 12[c], 16	HPLC	Total	PASI[13]	n/a
Chladek 1998	5, 9, 13	Enzyme ligand assay	Total	PASI[14]	n/a

TABLE 2 (Continued)

Study	MTX-PG measurement			Outcome	
	Timing (wk)	Method	PG species	Disease activity score	Toxicity
Chladek 2005	12[c]	Enzyme ligand assay	Total	PASI[14]	Liver
Chladek 2008	8, 16, 32	HPLC	Total	PASI[13,15]	n/a
Rahman 2014	12, 20	HPLC	Total	PGA[8]	n/a

Abbreviations: [A], abstract only; BM, bone marrow; CDAI, Crohn's disease activity index; cDMARD, conventional disease-modifying anti-rheumatic drugs; CNS, central nervous system; CRP, C-reactive protein; CZ, Czech Republic; DAS-28, Disease Activity Score of 28 joints; ESR, erythrocyte sedimentation rate; EULAR, DAS-based European League against Rheumatism response criteria; F, female; GI, gastrointestinal; IBDQ, Inflammatory Bowel Disease Questionnaire; m, months; mg/wk, milligram per week; *N*, number; n/a, not applicable; NL, The Netherlands; NSAIDs, nonsteroidal anti-inflammatory drugs; NZ, New Zealand; PGA, physician global assessment; RCT, randomized controlled trial; SJC, swollen joint count; TJC, tender joint count; wk, week; y, years; 5-ASA, 5-aminosalicyclic acid containing drugs.

[a]Mean ± standard deviation (unless not given), (range).

[b]Two articles of the same study. Mark is set by primary study.

[c]Time point of MTX-PG measurement is used for the analysis of the relationship with disease activity.

[d]Result of disease activity score at baseline for cohort studies and at the moment of analysis for cross-sectional studies. Scores used: for RA DAS-28; for JIA JADAS; for IBD the disease activity index.

Definitions of response:

[1]Delta used for (regression) analysis.

[2]Moderate or good EULAR response.

[3]Delta DAS-28 is greater than the median of 41%.

[4]Any improvement.

[5]Delta DAS-28 > 1.2.

[6]EULAR good response.

[7]Classified by (same treating) rheumatologist based on physician's global clinical assessment which included the patient history, joint examination and a comparison with the patient's condition at an earlier point in time, that is, a comparative temporal assessment.

[8]Physician global assessment of response scored by visual analogue scale (0 = high response, 10 = poor response). Responders had score of 2 cm or less.

[9]DAS-28 ≤ 3.2.

[10]IBDQ score ≥170 and discontinuation of prednisone.

[11]CDAI ≤ 150.

[12]Decrease in HBI of ≥3.

[13]PASI 50% improvement.

[14]Percentage of the initial PASI score.

[15]PASI 75% improvement.

[16]Three core sets improved by >30% and not more than 1 worsened by 30% (Reference: Gianninni 1997). Core sets: overall well-being, functional ability, no. of active joints, no. of joints with limited range of motion.

Definition of toxicity:

[a]Diarrhoea, nausea, vomiting, abdominal pain, mouth ulcers, decreased appetite, dyspepsia and/or stomatitis.

[b]Alanine aminotransferase 2–3 times upper level of normal (or above).

[c]Fatigue, dizziness, headache, sleeplessness, not feeling well, psychological complaints, anxiety, lethargy, loss of concentration, blurred vision, sleep disturbance, weepiness and/or forgetfulness.

[d]Leucocytes < 3×10^9/L and/or thrombocytes < 100×10^9/L.

[e]Elevation of aspartate aminotransferase above the upper limit of normal.

[f]Lymphocyte count < 0.9×10^9/L, granulocyte count < 1.5×10^9/L and/or thrombocyte count < 20×10^9/L.

[g]White blood cell count < 3.5×10^9/L, haemoglobin < 8 g/L, mean corpuscular volume > 120 fL.

[h]Elevation of aspartate aminotransferase or alanine aminotransferase above the upper limit of normal.

[i]Leucocytes < 4×10^9/L and/or thrombocytes < 150×10^9/L.

[j]Abnormalities of liver function necessitating dose reduction.

[k]Any difference in the complete blood cell count.

[l]Elevation of alkaline phosphatase, aspartate aminotransferase or alanine aminotransferase above the upper limit of normal.

[m]Alanine aminotransferase above upper level of normal and serum procollagen 3 above 4.2 μg/L.

[n]White blood cell < 4×10^9/L and/or neutrophil count < 1.5×10^9/L.

3.5.2 | Correlation coefficient

The summary Pearson correlation coefficient for MTX-PG_{total} and disease activity in the first 3 months of MTX use could be analysed in Ps patients only ($n = 86$; Figure 5A, Table S3). The included studies were of fair quality ($n = 3$),[29,45,48] 1 of poor quality[38] and 1 study was an abstract.[33] Although 3 of the 4 studies stemmed from the same author (Chladek et al.), there was

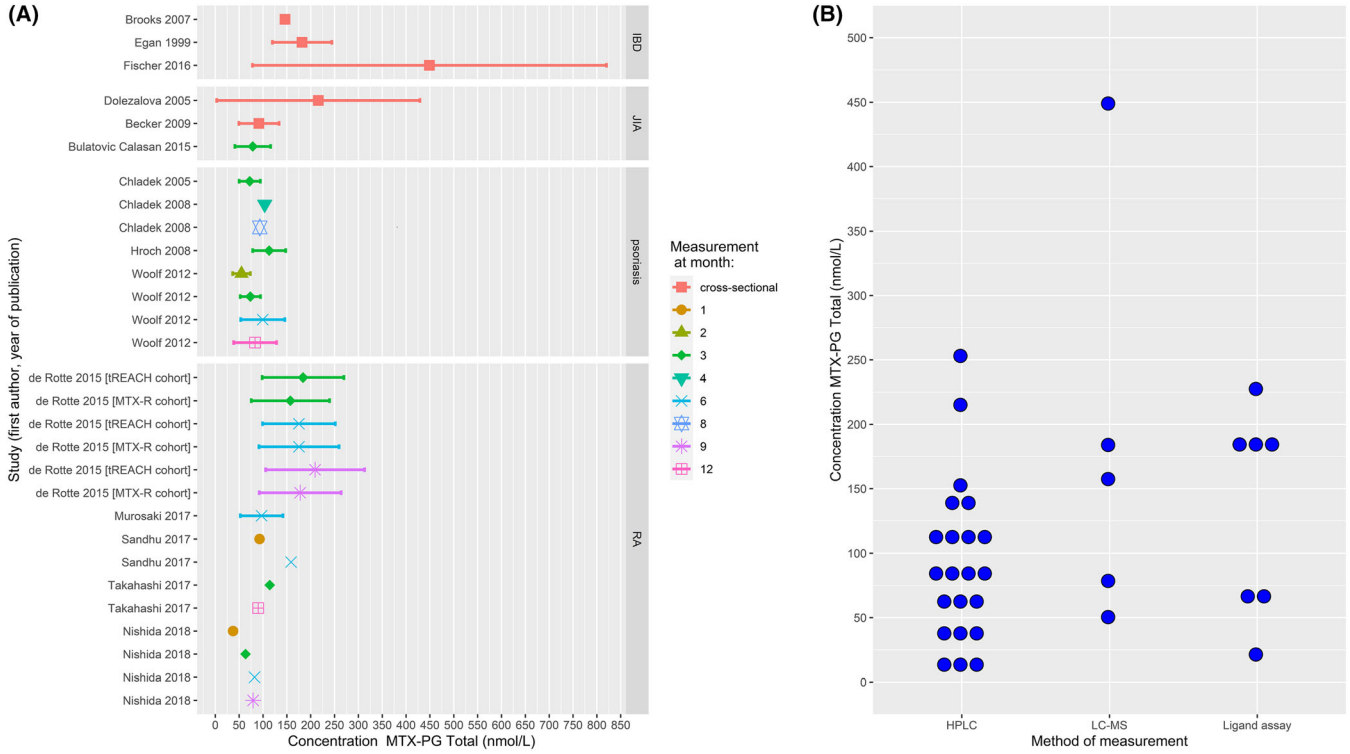

FIGURE 1 (A) Red blood cell MTX-PG_{total} concentration quantified independently of disease activity or toxicity. Mean ± standard deviation of red blood cell MTX-PG_{total} concentration (on the x-axis) independent of disease activity or toxicity shown per study cohort (on the y-axis) and timing of measurement (depicted by colour and symbol). Abbreviations: IBD, inflammatory bowel diseases; JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis. (B) Mean red blood cell MTX-PG_{total} concentration divided by method of measurement. Dots ($n = 34$) depict the mean concentration of a patient group (responders, nonresponder or a total patient group independently of disease activity/toxicity), derived from 21 studies in total. Each individual patient is represented only once. HPLC, high-performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry. Both LC–MS with (stable-isotope-labelled) internal standards or no internal standard were included in this category. Ligand assay includes both enzyme ligand assays as competitive protein binding assays.

considerable heterogeneity ($I^2 = 94.5\%$). The correlation coefficients were negative, leading to a significant summary Pearson correlation coefficient of −0.82 with a 95% CI of −0.976 to −0.102. This result means that higher MTX-PG_{total} concentrations are correlated with lower disease activity in Ps patients. In a study of 19 RA patients by Hobl et al., a significant negative correlation coefficient was also found between disease activity at 3 months and MTX-PG_2 species[49] (Figure S2).

In contrast to the psoriasis studies mentioned above and Hobl et al.,[49] Stamp et al.[45] analysed RA patients cross-sectionally and found a significant *positive* correlation for MTX-PG_{total} (Figure 5B) and MTX-PG species (Figure S2 and Table S3). This means that that higher MTX-PG concentrations were correlated with higher disease activity. Similarly, Brooks et al. also found a positive correlation of MTX-PG_4 and MTX-PG_5 with disease activity in their cross-sectional study of CD patients (data not shown).[44]

Cross-sectional studies by Becker et al. (JIA, data not shown), Fischer/Shivi et al. (IBD, secondary report of Fisher) and Woolf et al. (Ps) did not find significant correlations between disease activity and various MTX-PG species (Figure S2).[36,37,46,48]

3.5.3 | Odds ratio

In 3 RA studies of Dervieux et al., an odds ratio was computed to analyse the association between disease activity and MTX-PG concentrations. The increase of MTX-PG_3 levels above 132 nmol/L was associated with a 25.5-fold higher likelihood of response (95% CI 4.7–139.2; $P < .001$).[34] Patients with MTX-PG_{total} levels <60 nmol/L were 4.4-fold more likely to be nonresponders ($P < .001$).[50] Furthermore, patients with MTX-PG_{total} < 20 nmol/L at month 3 after MTX start were more likely to experience nonresponse (odds ratio 8.4, 95% CI 1.4–52.3; $P = .02$).[47]

3.5.4 | MD

Figure 6 shows the forest plot of the MD of MTX-PG_{total} concentration between responders and nonresponders at months 1 to 3 (A) and from months 4 onwards (B).[12,13,29,37,38] Figure 6A shows 238 responders and 106 nonresponders in total for JIA, psoriasis and RA. The summary MD of 5.3 nmol/L between responders and

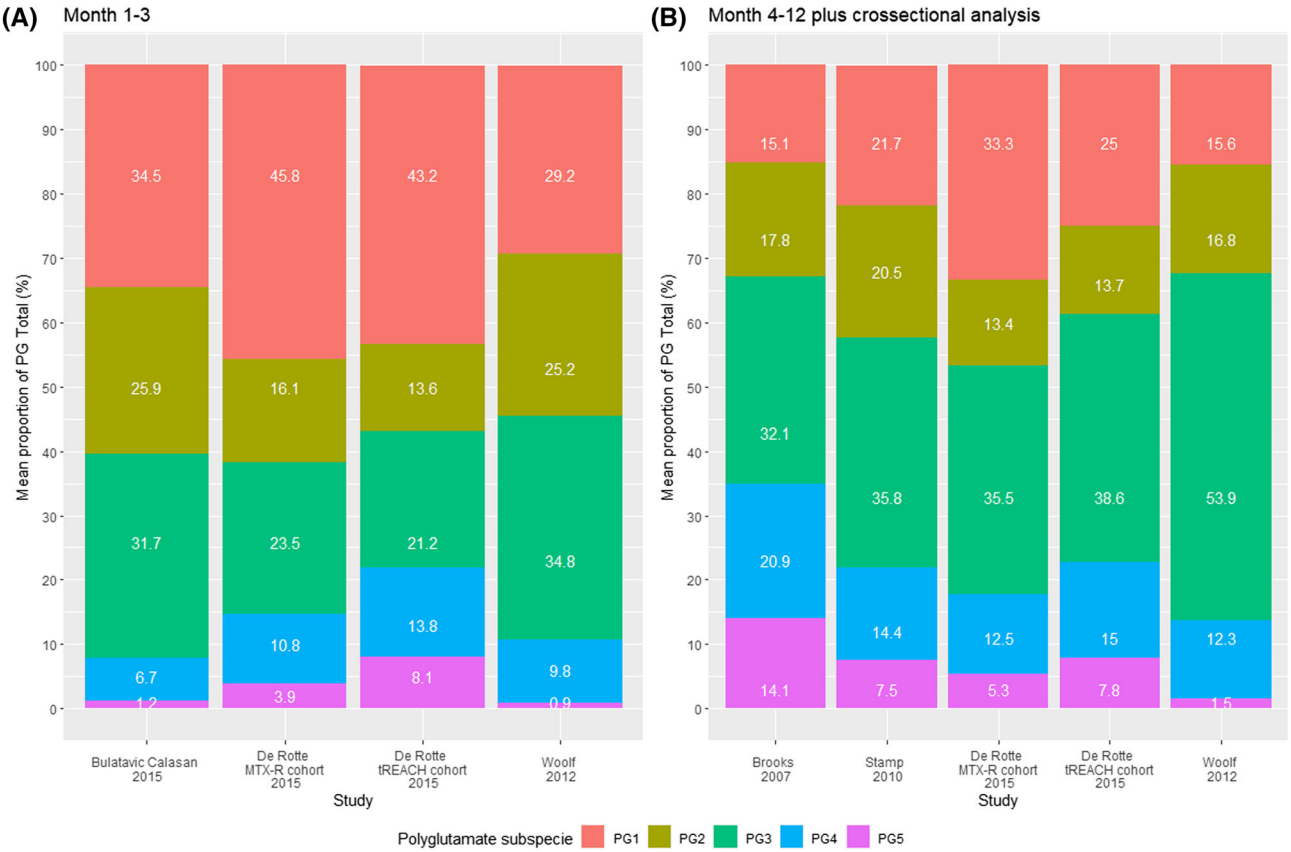

FIGURE 2 Proportion polyglutamate subspecies of MTX-PG_{total} concentration measured independent of disease activity and/or toxicity. (A) Weighted mean proportion of concentrations measured during months 1–3 since start MTX. *P* of random-effects (RE) model for rheumatoid arthritis studies = .05. *P* of RE model for all studies < .01. (B) Weighted mean proportion of concentrations measured during months 4–12 since start MTX or in cross-sectional analysis. *P* of RE model for rheumatoid arthritis studies = <.01. *P* of RE model for all studies = <.01.

nonresponders was statistically significant (*P* < .01). This result means that responders have a 5.3 nmol/L higher concentration of MTX-PG_{total} than nonresponders. The model showed no significant differences between IMIDs and no heterogeneity.

Figure 6B shows 323 responders and 94 nonresponders across IMIDs from month 4 onward. The summary MD of 14.5 nmol/L between responders and nonresponders was nonsignificant (*P* = .11). The summary MD for RA studies only was significant (MD 29.6 nmol/L, *P* < .01) but not for Ps/AD and not for the single JIA and IBD studies. There was heterogeneity both in the meta-analysis of all studies as well as in the meta-analysis of Ps/AD and RA studies separately. The funnel plot (Figure S3) showed some publication bias for small studies but not for studies with a larger sample size and lower standard errors. Summary MD of MTX-PG species wAS positive and significant for MTX-PG_2, MTX-PG_4 and MTX-PG_5 after 4 months (Table S4).

It is noteworthy that IBD patients (Egan et al.) and AD patients (Rahman et al.) could only be included in the analysis of MTX-PG_{total} MD between 4 and 12 months of MTX use.[40,51] Therefore, the results of the overall meta-analysis cannot be extrapolated to IBD and AD patients.

3.6 | Relationship between toxicity and MTX-PG concentration

A meta-analysis of the relationship between toxicity and MTX-PG concentration was not performed due to limited and heterogeneous data. Pooling of different toxicity data was not possible due to multiple studies without numeric data (particularly without standard deviations) for different polyglutamate species and different categories of toxicity.

Overall, most studies showed no relationship between any form of toxicity and MTX-PG concentration. There was no relationship found between hepatotoxicity and MTX-PG_3/MTX-PG_{total} concentration in 6 studies.[12,13,26,37,39,45] Conversely, Chladek et al. did show that MTX-PG level at 3 months were higher in Ps patients experiencing hepatotoxicity (MD MTX-PG_{total} 35.0 nmol/L, 95% CI 10.5–59.5 nmol/L).[38] Takahashi et al. found higher concentrations of MTX-PG_{total} in Japanese RA patients with hepatotoxicity and advised not exceeding MTX-PG_{total} concentration beyond 105 nmol/L.[41] Three studies in psoriasis, RA and JIA did not demonstrate a relationship between bone marrow toxicity and MTX-PG.[12,13,37] On the contrary, Stamp et al. showed that lower

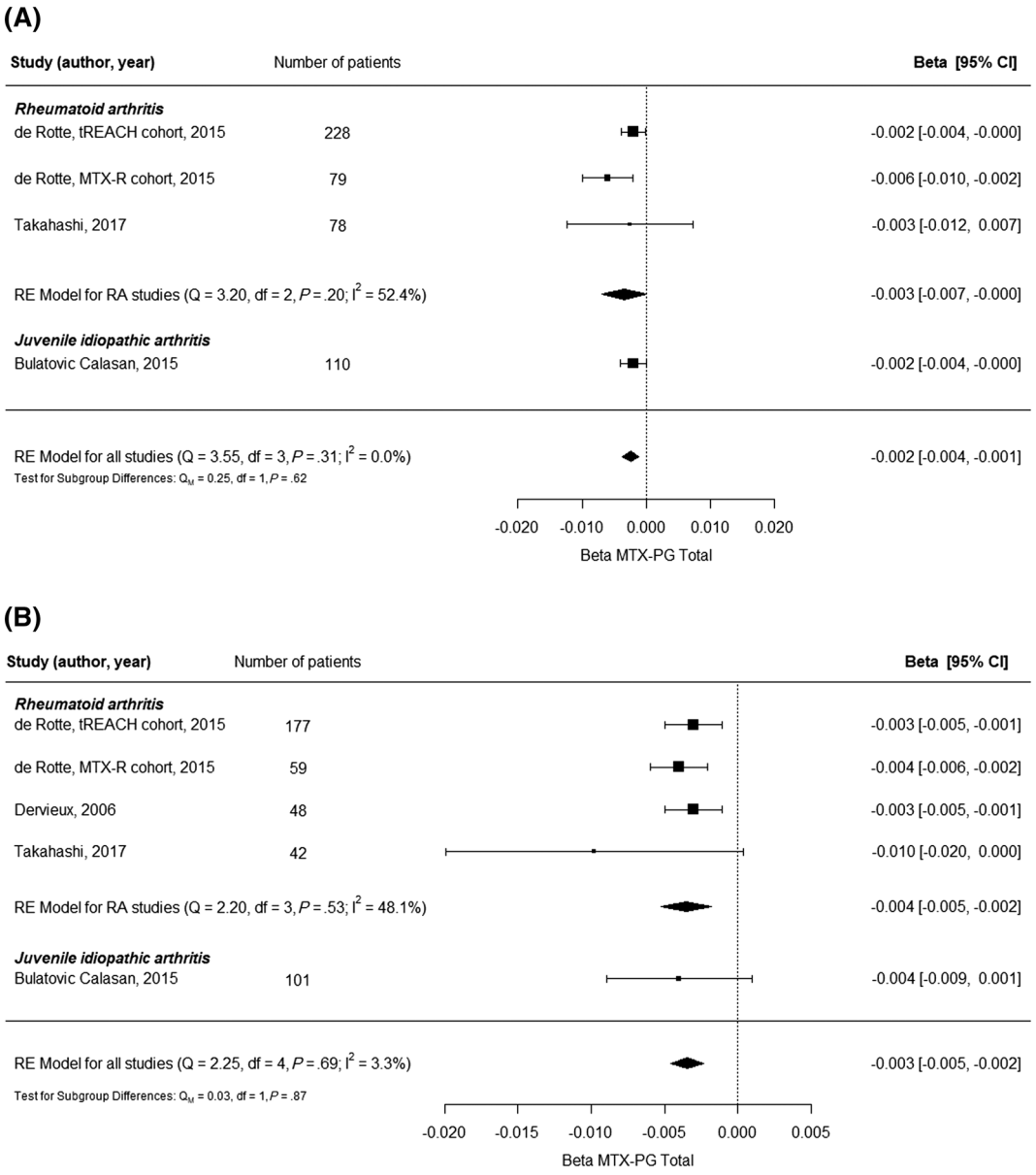

FIGURE 3 Forest plot of β regression coefficient of MTX-PG_{total} concentration related to disease activity. (A) Pooled β with 95% confidence interval (CI) of concentrations measured during months 1–3 since start MTX. *P* of random-effects (RE) model for rheumatoid arthritis studies = .05. *P* of RE model for all studies <.01. (B) Pooled β with 95% CI of concentrations measured during months 4–12 since start MTX or in cross-sectional analysis. *P* of RE model for rheumatoid arthritis studies <.01. *P* of RE model for all studies <.01. See Table S2 for details of included regression coefficients.

haemoglobin and higher mean corpuscular volume were associated with higher MTX-PG concentrations.[45] Six studies[12,13,26,37,45,52] reported no relationship between gastrointestinal toxicity and MTX-PG concentration with the exception of Brooks et al., showing that higher MTX-PG_4 and MTX-PG_5 were associated with gastrointestinal side effects.[44] Also, no relationship was found between MTX-PG concentrations and central nervous system side effects in RA.[12,45] Finally, studies analysing toxicity as a general category did not reveal a relationship between overall toxicity and MTX-PG.[12,13,46,47]

4 | DISCUSSION

In this meta-analysis, we found that higher erythrocyte MTX-PG_{total} concentration was associated with lower disease activity in RA, JIA and psoriasis. Furthermore, we demonstrated that MTX-PG concentrations show considerable interindividual variation. These findings are an important step towards implementation of TDM for MTX across IMIDs, since interindividual variability and a relationship between drug concentrations and disease activity are the 2 prerequisites for TDM use in clinical practice. To the best of our knowledge,

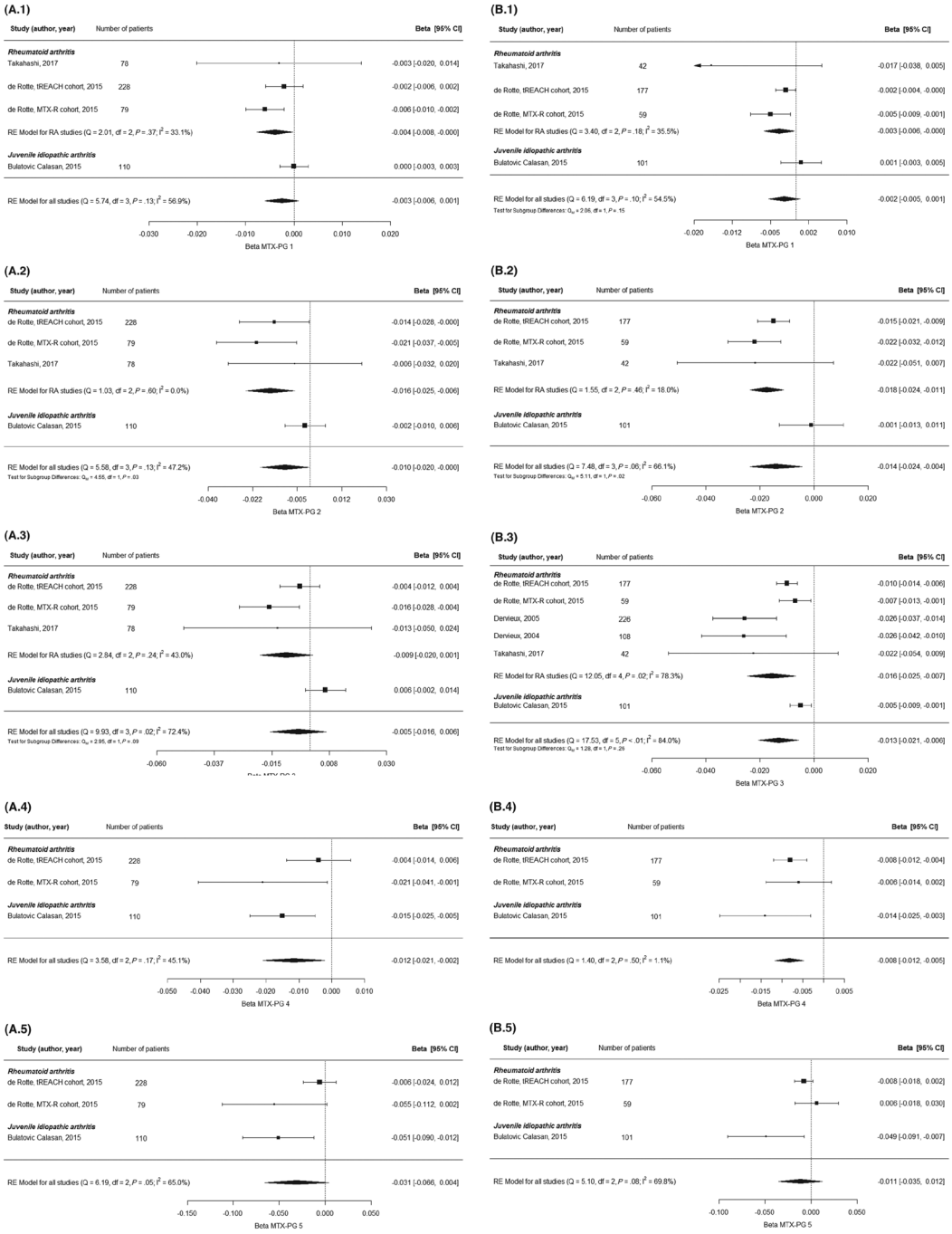

FIGURE 4 Forest plot of β regression coefficient of MTX-PG_{1-5} concentration related to disease activity. (A) Pooled β with 95% confidence interval (CI) of concentrations measured during months 1–3 since start MTX. *P* of random-effects (RE) model for rheumatoid arthritis included studies of MTX-PG_1 $P = .04$; MTX-PG_2 $P < .01$; MTX-PG_3 $P = .08$. *P* of RE model for all included studies of MTX-PG_1 $P = .15$; MTX-PG_2 $P = .05$; MTX-PG_3 $P = .41$; MTX-PG_4 $P = .02$; MTX-PG_5 $= .08$. (B) Pooled β with 95% CI of concentrations measured during months 4–12 since start MTX or in cross-sectional analysis. *P* of RE model for rheumatoid arthritis included studies of MTX-PG_1 $P = .03$; MTX-PG_2 $P < .01$; MTX-PG_3 $P < .01$. *P* of RE model for all included studies of MTX-PG_1 $P = .15$; MTX-PG_2 $P < .01$; MTX-PG_3 $P < .01$; MTX-PG_4 $P < .01$; MTX-PG_5 $= .35$. See Table S2 for details of included regression coefficients.

this is the first meta-analysis on the utility of erythrocyte MTX-PG TDM in low-dose MTX treatment across IMIDs, providing comprehensive and solid quantitative evidence on the relationship of higher erythrocyte MTX-PG concentrations with lower disease activity.

Because higher erythrocyte MTX-PG_{total} and higher MTX-PG species were related to lower disease activity across IMIDs with high interindividual variation, MTX-PG_{total} or 1 of the species can and should be chosen for TDM implementation across IMIDs. MTX-PG_1, whose concentrations are influenced by the plasma compartment and

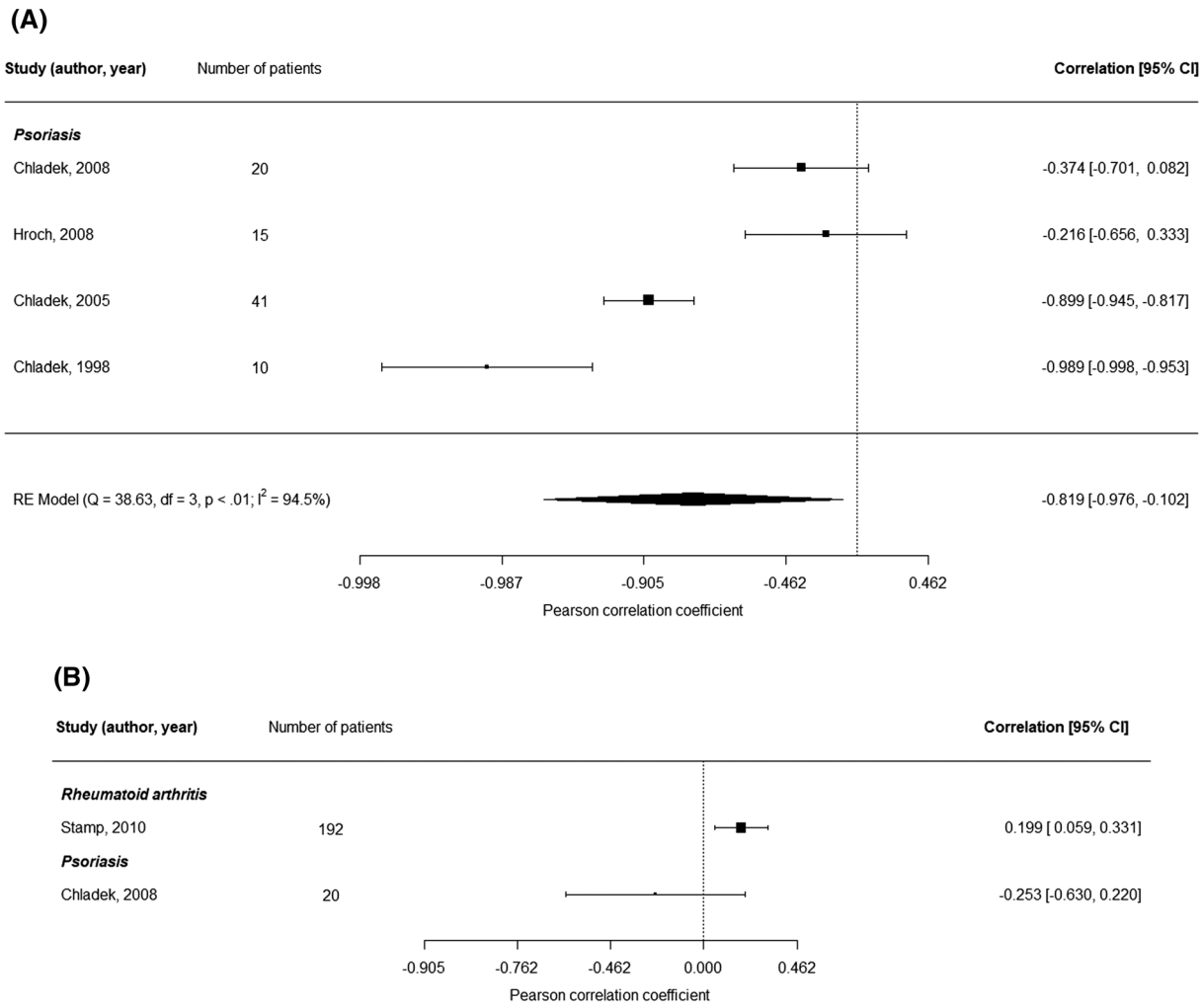

FIGURE 5 Forest plot of Pearson correlation coefficient with 95% confidence interval (CI) of MTX-PG_{total} in relation to disease activity. (A) Pooled coefficient with 95% CI of concentrations measured during months 1–3 since start MTX. (B) Weighted coefficient with 95% CI of concentrations measured during months 4–12 since start MTX or in cross-sectional analysis. See Table S3 for details of included correlation coefficients.

the efflux from erythrocytes leading to larger *intra*patient variability[53] and MTX-PG_5, which is present in very low concentrations, are therefore less appropriate as TDM tools.[25] MTX-PG_{2-4} species are retained intracellularly and are therefore likely to be the most clinically relevant species and henceforth the most appropriate TDM tools. Also, the summary β in our meta-analysis was significant for the measurement of MTX-PG_{2-4} after 4 months of MTX use (Figure 4B). Of these species, MTX-PG_3 is the most abundant (Figure 2) and as such could be selected as a TDM tool. We suggest using MTX-PG_{total} for the purpose of TDM in MTX treatment, although MTX-PG_3 could be considered as well.

Alongside the selection of the MTX-PG species to be measured for TDM, choosing an appropriate MTX-PG cut-off value differentiating between responders and nonresponders is also desirable for TDM application in clinical practice in order to guide physician's decisions. Such a cut-off value could be used in the following fashion: In case a patient, having active disease, does not achieve a predetermined MTX-PG concentration after 3–6 months of treatment with a maximum (tolerable) dose, such a patient would switch to another or additional treatment (such as biologics), with the aim of reaching faster disease control. In other situations, the dose could be escalated (in face of lower MTX dose and failure to achieve target MTX-PG concentration) or adherence to MTX could be questioned (in face of very low or unquantifiable MTX-PG concentration).

Identifying a clinically relevant MTX-PG cut-off value was beyond the scope of this meta-analysis which focused on the relationship of the MTX-PG concentration and disease activity and toxicity. Of the included studies, 12 studies (10 RA, 1 JIA, 1 Ps/AD) suggested cut-off MTX-PG concentrations for reaching response or remission (Table S5). The cut-off values for MTX-PG_{total} ranged from 27.3 to 83.3 nmol/L.[12,41,42,47,51,54] The variability of concentrations and cut-off values between studies could be due to a small sample size of responders and nonresponders, usage of different MTX doses, different routes of administration, different timing of MTX-PG measurement or unknown compliance to MTX treatment. However, we also showed that the variability of MTX-PG concentrations

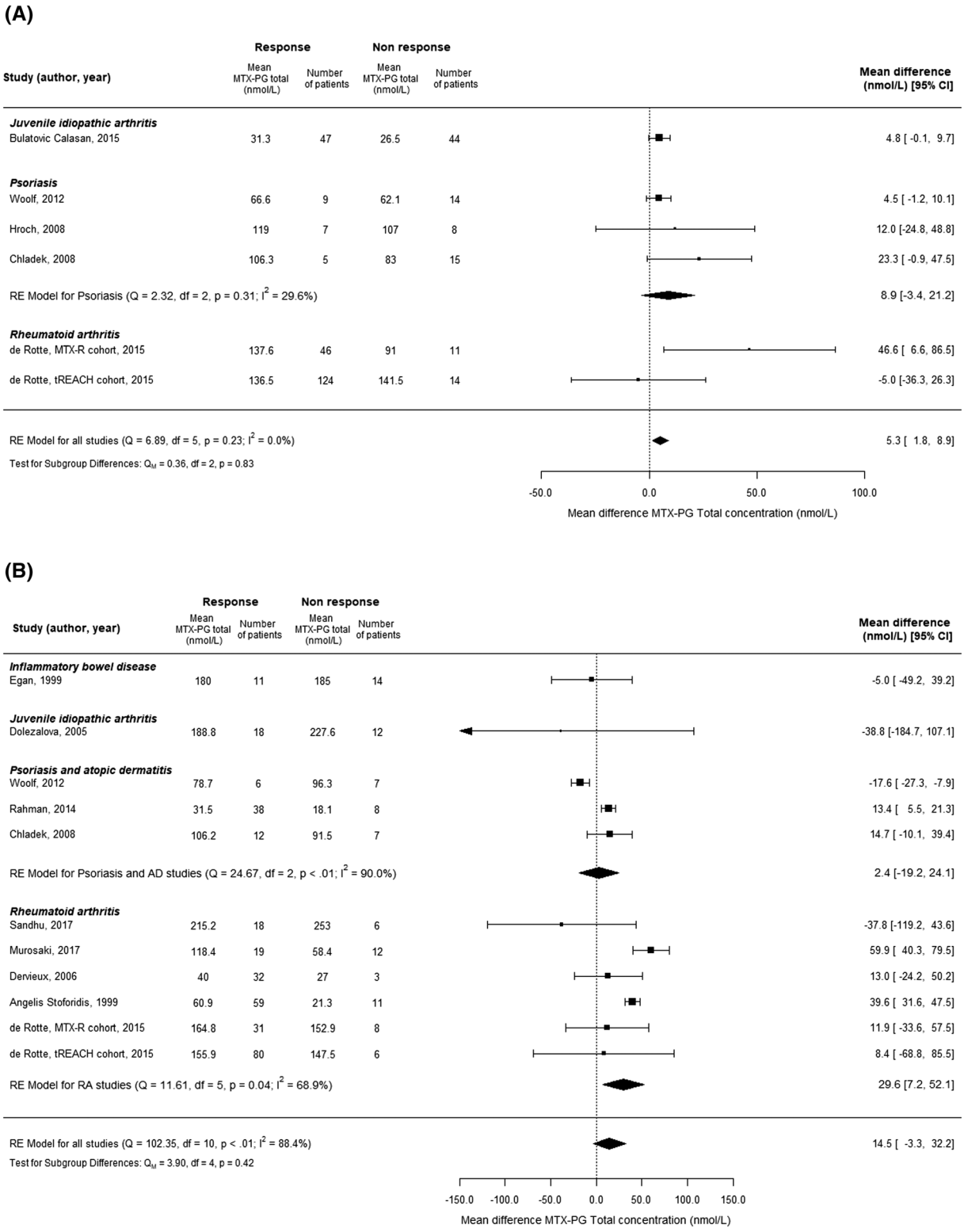

FIGURE 6 Forest plot of mean difference (MD) of MTX-PG_{total} concentration in nmol/L for responders *vs.* nonresponders. (A) MD with 95% CI of weighted mean concentrations measured during months 1–3 since start MTX. *P* of random-effects (RE) model for included studies in patients with psoriasis, $P = .16$. *P* of RE model for all included studies, $P < .01$. (B) MD with 95% CI of weighted mean concentrations measured during months 4–12 since start MTX or in cross-sectional analysis. *P* of RE model for included studies in patients with psoriasis and/or atopic dermatitis, $P = .83$. *P* of RE model for included studies in patients with rheumatoid arthritis, $P < .01$. *P* of RE model for all included studies, $P = .11$.

seemed independent of disease type, timing of measurement or the laboratory technique (Figure 1). It is noteworthy that sample preparation and analysis with immune (ligand) assays have to be performed carefully to prevent positive bias based on interference from folates and MTX metabolites,[25] whereas both LC–MS/MS and high-performance LC methods are accurate and have low limits of quantification.[55] These methods are readily available in clinical chemistry laboratories and pharmacies and the measurement of erythrocyte MTX-PG is affordable (estimated cost in the Netherlands of €35–60 per sample, comparable to measuring methylmalonic acid [personal communication]), which facilitates clinical applicability of TDM using MTX-PG.

The secondary aim was to analyse the relationship between various forms of MTX toxicity and MTX-PG levels. However, due to limited and heterogeneous data, no definite or reliable conclusion could be drawn on this relationship. There was no consistent trend showing more adverse events in patients with higher MTX-PG concentrations. This means that, based on our meta-analysis, erythrocyte MTX-PG TDM cannot be applied for modification of clinical decisions in case of MTX toxicity.

A strength of our meta-analysis is the inclusion of various IMIDs in which treatment with MTX plays a key role. By dichotomizing disease-specific activity scores to the universal *response vs. nonresponse*, we were able to pool the available data therefore increasing power. Including different IMIDs did not increase the heterogeneity (I^2 of models including studies of all IMIDs are smaller than I^2 of disease-specific models) as the diagnosis did not appear to influence MTX-PG concentration levels (Figure 1A).

Our meta-analysis has some limitations. First, we chose to pool effect measures of several time points within 1 study and calculated a weighted mean ± SD for the period between months 1–3 and months 4–12. This could lead to a nonsignificant effect measure of merged time points, whereas in the original study the effect measure was significant for 1 specific time point. However, the decision to summarize several time points provided more data and enabled analysis of multiple studies together, which lead to more power. Second, we were unable to perform subgroup analysis with variables such as the route of administration and MTX dose or to exclude concomitant steroid users. The first 2 could influence the level of the MTX-PG concentrations and the latter could influence the disease activity score as well as the MTX-PG concentrations.[56–58] To minimize the influence of concomitant drug use, we excluded studies with patients on biologic medications. Third, erythrocytes might serve as a surrogate cell type for measurement of MTX-PG levels, since erythrocytes harbour some residual reduced folate carrier and folylpolyglutamate synthetase activity; however, they are enucleated and are not proliferating as opposed to peripheral blood mononuclear cells (PBMCs). PBMCs could reflect MTX pharmacokinetics more accurately than the erythrocytes; however, MTX-PG measurements in PBMCs are challenging and as of yet not readily available.[59,60] Furthermore, the results of this meta-analysis cannot be extrapolated to IBD and AD patients as only 1 IBD study (Egan et al.) and 1 AD study (Rahman et al. combined with psoriasis) were included in a single sub-meta-analysis of MTX-PG difference between 4 and 12 months of MTX use.[40,51]

To satisfy the ultimate goal of implementing TDM of MTX in order to guide treatment decisions, future studies should ideally be prospective cohorts with longitudinal follow-up of disease activity (as continuous and dichotomous outcomes), toxicity and drug levels. The following set of (minimal) requirements should be satisfied: reporting of MTX-PG concentrations, sample size and mean dose (±SD) per time point from the first month of MTX use onwards, standardized βs, odds ratios and target MTX-PG concentrations in relation to disease activity. Such pharmacodynamic studies should preferably be combined with pharmacokinetic data retrieval, including measurements of erythrocyte folate levels and analysis of (epi)genetic markers. Following the example of Syversen et al. who performed a randomized controlled trial of infliximab TDM in patients with chronic IMIDs,[61] the abovementioned pharmacokinetic and pharmacodynamic data should ideally be obtained across IMIDs.

5 | CONCLUSION

In this comprehensive meta-analysis, we showed that higher concentrations of erythrocyte MTX-PG_{total} were associated with lower disease activity in RA, JIA and psoriasis, accompanied by considerable interindividual variation. These findings are an important step towards the use of TDM for MTX treatment across IMIDs. Due to large heterogeneity of currently available studies, future studies on MTX pharmacodynamics should satisfy a minimal set of requirements, in order to bring TDM of MTX closer to common practice.

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COMPETING INTERESTS

None of the authors have conflicts of interest.

CONTRIBUTORS

R.d.J., M.N., M.B., M.v.d.M. and R.H. designed the meta-analysis. M.v.d.M., R.H. and M.B. conducted the statistical analyses. M.v.d.M., R.H. and M.B. wrote the manuscript. All authors reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Almutairi KB, Nossent JC, Preen DB, Keen HI, Inderjeeth CA. The prevalence of rheumatoid arthritis: a systematic review of population-based studies. *J Rheumatol*. 2021;48(5):669-676. doi:10.3899/jrheum.200367
2. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2017;390(10114):2769-2778. doi:10.1016/S0140-6736(17)32448-0
3. Hay RJ, Johns NE, Williams HC, et al. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *J Invest Dermatol*. 2014;134(6):1527-1534. doi:10.1038/jid.2013.446
4. Smolen JS, Landewé RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis*. 2020;79(6):S685-S699.
5. Lundberg IE, Tjärnlund A, Bottai M, et al. EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups HHS public access. *Ann Rheum Dis*. 2017;76(12):1955-1964. doi:10.1136/annrheumdis-2017-211468
6. Wollenberg A, Barbarot S, Bieber T, et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part II. *J Eur Acad Dermatol Venereol*. 2018;32(6):850-878. doi:10.1111/jdv.14888
7. Nast A, Smith C, Spuls PI, et al. EuroGuiDerm guideline on the systemic treatment of *Psoriasis vulgaris*—part 1: treatment and monitoring recommendations. *J Eur Acad Dermatol Venereol*. 2020;34(11):2461-2498. doi:10.1111/jdv.16915
8. Torres J, Bonovas S, Doherty G, et al. ECCO guidelines on therapeutics in Crohn's disease: medical treatment. *J Crohns Colitis*. 2020;14(1):4-22. doi:10.1093/ecco-jcc/jjz180
9. Rohr MK, Mikuls TR, Cohen SB, Thorne JC, O'Dell JR. Underuse of methotrexate in the treatment of rheumatoid arthritis: a national analysis of prescribing practices in the US. *Arthritis Care Res*. 2017;69(6):794-800. doi:10.1002/acr.23152
10. Ensom H, Davis GA, Cropp CD, Ensom RJ. Clinical pharmacokinetics in the 21st century. *Clin Pharmacokinet*. 1998;34(4):265-279. doi:10.2165/00003088-199834040-00001
11. Den Boer E, Meesters RJW, Van Zelst BD, et al. Measuring methotrexate polyglutamates in red blood cells: a new LC-MS/MS-based method. *Anal Bioanal Chem*. 2013;405(5):1673-1681. doi:10.1007/s00216-012-6581-7
12. De Rotte MCFJ, Den Boer E, De Jong PHP, et al. Methotrexate polyglutamates in erythrocytes are associated with lower disease activity in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2015;74(2):408-414. doi:10.1136/annrheumdis-2013-203725
13. Bulatović Ćalasan M, Den Boer E, De Rotte MCFJ, et al. Methotrexate polyglutamates in erythrocytes are associated with lower disease activity in juvenile idiopathic arthritis patients. *Ann Rheum Dis*. 2015;74(2):402-407. doi:10.1136/annrheumdis-2013-203723
14. Muller IB, Hebing RF, Jansen G, et al. Personalized medicine in rheumatoid arthritis: methotrexate polyglutamylation revisited. *Expert Rev Precis Med Drug Dev*. 2018;3(6):331-334. doi:10.1080/23808993.2018.1517025
15. Mohamed HJ, Sorich MJ, Kowalski SM, et al. The role and utility of measuring red blood cell methotrexate polyglutamate concentrations in inflammatory arthropathies—a systematic review. *Eur J Clin Pharmacol*. 2015;71(4):411-423. doi:10.1007/s00228-015-1819-x
16. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. 2014;14(1):1, 135-13. doi:10.1186/1471-2288-14-135
17. Cochrane. Obtaining standard deviations from standard errors and confidence intervals for group means. https://handbook-5-1.cochrane.org/chapter_7/7_7_3_2_obtaining_standard_deviations_from_standard_errors_and.htm
18. Perumal K. Formula for calculate standard error from confidence interval? 2018. https://handbook-5-1.cochrane.org/chapter_7/7_7_3_2_obtaining_standard_deviations_from_standard_errors_and.htm
19. Huwaldt JA. Plot digitizer (Version 2.6.9). Source Forge. Download from https://sourceforge.net/projects/plotdigitizer/. Source Forge; 2014.
20. NHLBI. *Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies*. Bethesda, MD: National Institutes of Health, Department of Health and Human Services; 2014. https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools
21. Irvine EJ, Feagan B, Rochon J, et al. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. *Gastroenterology*. 1994;106(2):287-296. doi:10.1016/0016-5085(94)90585-1
22. Vermeire S, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation between the Crohn's disease activity and Harvey-Bradshaw indices in assessing Crohn's disease severity. *Clin Gastroenterol Hepatol*. 2010;8(4):357-363. doi:10.1016/j.cgh.2010.01.001
23. Fransen J, van Riel PLCM. The Disease Activity Score and the EULAR response criteria. *Clin Exp Rheumatol*. 2005;23(5 Suppl 39):S93-S99.
24. Rupinski MT, Dunlap WP. Approximating Pearson product-moment correlations from Kendall's tau and Spearman's rho. *Educ Psychol Meas*. 1996;56(3):419-429. doi:10.1177/0013164496056003004
25. Den Boer E, De Rotte MCJF, Pluijm SMF, Heil SG, Hazes JM, De Jonge R. Determinants of erythrocyte methotrexate polyglutamate levels in rheumatoid arthritis. *J Rheumatol*. 2014;41(11):2167-2178. doi:10.3899/jrheum.131290
26. Angelis-Stoforidis P, Vajda FJE, Christophidis N. Methotrexate polyglutamate levels in circulating erythrocytes and polymorphs correlate with clinical efficacy in rheumatoid arthritis. *Clin Exp Rheumatol*. 1999;17(3):313-320.
27. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw*. 2010;36(3):1-48. doi:10.18637/jss.v036.i03
28. Deeks J, Higgins J, Altman D. Chapter 10: Analysing data and undertaking meta-analyses. In: *Cochrane Handbook for Systematic Reviews of Interventions version 6.2*; 2021. https://training.cochrane.org/handbook/current/chapter-10
29. Hroch M, Chladek J, Simkova M, Vaneckova J, Grim J, Martinkova J. A pilot study of pharmacokinetically guided dosing of oral methotrexate in the initial phase of psoriasis treatment. *J Eur Acad Dermatol Venereol*. 2008;22(1):19-24.
30. Fisher RA. Sample, on the "probable error" of a coefficient of correlation deduced from a small. *Metron*. 1921;1(1):3-32.
31. Gridneva G, Muraviev Y, Lila A, Baymeeva N. The relationship between the effectiveness of methotrexate (MTX) in rheumatoid arthritis (RA) and mtx polyglutamates levels (MTXPGS) in red blood cells by tandem chromatomass spectrometry (pilot study). *Ann Rheum Dis*. 2019;78:747.
32. Nishida M, Tsuji G, Takahashi M, et al. Rapid elevation of erythrocyte methotrexate-polyglutamate3 (MTX-PG3) levels related to the efficacy of MTX in patients with rheumatoid arthritis (RA). *Ann Rheum Dis*. 2018;77:980-981.
33. Chladek J, Grim J, Martinkova J, Simkova M, Vaneckova J. Low-dose methotrexate pharmacokinetics and pharmacodynamics in the therapy of severe psoriasis. *Basic Clin Pharmacol Toxicol*. 2005;96(3):247-248. doi:10.1111/j.1742-7843.2005.pto960318.x
34. Dervieux T, Zablocki R, Kremer J. The sequential addition of glutamic residues on methotrexate selectively potentiates the efficacy of this prodrug in rheumatoid arthritis. *Arthritis Rheum*. 2009;60(S10):Abstractnr 399.

35. Dervieux T, Matsutani M, Stimson D, Barry M, McGuyer C. Preliminary estimates and significance of red blood cell methotrexate polyglutamate measurements in the rheumatology practice setting. *Arthritis Rheum*. 2009;60(S10):Abstract nr1619.
36. Becker ML, Van Haandel L, Lasky AS, et al. Analysis of intracellular methotrexate polyglutamates in juvenile idiopathic arthritis. *Arthritis Rheumatol*. 2009;60:228.
37. Woolf RT, West SL, Arenas-Hernandez M, et al. Methotrexate polyglutamates as a marker of patient compliance and clinical response in psoriasis: a single-centre prospective study. *Br J Dermatol*. 2012; 167(1):165-173. doi:10.1111/j.1365-2133.2012.10881.x
38. Chládek J, Simková M, Vanecková J, et al. The effect of folic acid supplementation on the pharmacokinetics and pharmacodynamics of oral methotrexate during the remission-induction period of treatment for moderate-to-severe plaque psoriasis. *Eur J Clin Pharmacol*. 2008; 64(4):347-355. doi:10.1007/s00228-007-0442-x
39. Sandhu A, Dhir V, Bhatnagar A, Dhawan V, Kaur J, Sood A. High methotrexate triglutamate level is an independent predictor of adverse effects in Asian Indian rheumatoid arthritis patients—a preliminary study. *Ther Drug Monit*. 2017;39(2):157-163. doi:10.1097/FTD.0000000000000375
40. Egan LJ, Sandborn WJ, Tremaine WJ, et al. A randomized dose-response and pharmacokinetic study of methotrexate for refractory inflammatory Crohn's disease and ulcerative colitis. *Aliment Pharmacol Ther*. 1999;13(12):1597-1604. doi:10.1046/j.1365-2036.1999.00667.x
41. Takahashi C, Kaneko Y, Okano Y, et al. Association of erythrocyte methotrexate-polyglutamate levels with the efficacy and hepatotoxicity of methotrexate in patients with rheumatoid arthritis: a 76-week prospective study. *RMD Open*. 2017;3:e000363. doi:10.1136/rmdopen-2016-000363
42. Murosaki T, Nagatani K, Sato T, et al. Prediction of the therapeutic response to methotrexate at 24 weeks by methotrexate–polyglutamates concentration in erythrocytes at 8 weeks in patients with rheumatoid arthritis. *Mod Rheumatol*. 2017;27(3):411-416. doi:10.1080/14397595.2016.1208137
43. Dervieux T, Lein DO, Marcelletti J, et al. HPLC determination of erythrocyte methotrexate polyglutamates after low-dose methotrexate therapy in patients with rheumatoid arthritis. *Clin Chem*. 2003; 49(10):1632-1641. doi:10.1373/49.10.1632
44. Brooks AJ, Begg EJ, Zhang M, Frampton CM, Barclay ML. Red blood cell methotrexate polyglutamate concentrations in inflammatory bowel disease. *Ther Drug Monit*. 2007;29(5):619-625. doi:10.1097/FTD.0b013e31811f39bb
45. Stamp LK, O'Donnell JL, Chapman PT, et al. Methotrexate polyglutamate concentrations are not associated with disease control in rheumatoid arthritis patients receiving long-term methotrexate therapy. *Arthritis Rheum*. 2010;62(2):359-368. doi:10.1002/art.27201
46. Fischer M, Siva S, Cook GK, Jones DR, Fadda HM. Methotrexate polyglutamate monitoring in patients with Crohn's disease. *Clin Pharmacol Drug Dev*. 2017;6(3):240-245. doi:10.1002/cpdd.279
47. Dervieux T, Greenstein N, Kremer J. Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis. *Arthritis Rheum*. 2006;54(10):3095-3103. doi:10.1002/art.22129
48. Shivi S, Gwendolyn C, Hala F, Vemulapalli K, David J, Monika F. Red blood cell and white blood cell methotrexate polyglutamate concentrations in patients with Crohn's disease. *Inflamm Bowel Dis*. 2014;20: S52 doi:10.1097/01.MIB.0000456799.78078.b6
49. Hobl E-L, Jilma B, Erlacher L, et al. A short-chain methotrexate polyglutamate as outcome parameter in rheumatoid arthritis patients receiving methotrexate. *Clin Exp Rheumatol*. 2012;30(2): 156-163.
50. Dervieux T, Furst D, Lein DO, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study. *Ann Rheum Dis*. 2005; 64(8):1180-1185. doi:10.1136/ard.2004.033399
51. Rahman SI, Siegfried E, Flanagan KH, Armbrecht ES. The methotrexate polyglutamate assay supports the efficacy of methotrexate for severe inflammatory skin disease in children. *J am Acad Dermatol*. 2014;70(2):252-256. doi:10.1016/j.jaad.2013.10.001
52. Doležalová P, Krijt J, Chládek J, Němcová D, Hoza J. Adenosine and methotrexate polyglutamate concentrations in patients with juvenile arthritis. *Rheumatology*. 2005;44(1):74-79.
53. Blits M, Jansen G, Assaraf YG, et al. Methotrexate normalizes up-regulated folate pathway genes in rheumatoid arthritis. *Arthritis Rheum*. 2013;65(11):2791-2802. doi:10.1002/art.38094
54. Dervieux T, Furst D, Lein DO, et al. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. *Arthritis Rheum*. 2004;50(9):2766-2774. doi:10.1002/art.20460
55. Karami F, Ranjbar S, Ghasemi Y, Negahdaripour M. Analytical methodologies for determination of methotrexate and its metabolites in pharmaceutical, biological and environmental samples. *J Pharm Anal*. 2019;9(6):373-391. doi:10.1016/j.jpha.2019.06.001
56. King DJ, Brunton J, Barr RD. The influence of corticosteroids on human erythropoiesis. an in vivo study. *Am J Pediatr Hematol Oncol*. 1988;10(4):313-315. doi:10.1097/00043426-198824000-00008
57. Hebing R, Mahmoud S, Lin M, et al. Erythrocyte methotrexate polyglutamates are substantially higher after subcutaneous methotrexate treatment in rheumatoid arthritis patients in the first months of treatment. *Arthritis Rheumatol*. 2021;73(Suppl 10).
58. Bianchi G, Caporali R, Todoerti M, Mattana P. Methotrexate and rheumatoid arthritis: current evidence regarding subcutaneous versus oral routes of administration. *Adv Ther*. 2016;33(3):369-378. doi:10.1007/s12325-016-0295-8
59. Korell J, Stamp LK, Barclay ML, et al. A population pharmacokinetic model for low-dose methotrexate and its polyglutamated metabolites in red blood cells. *Clin Pharmacokinet*. 2013;52(6):475-485. doi:10.1007/s40262-013-0052-y
60. Van Haandel L, Steven LJ, Becker M. Measurement of methotrexate metobolites in peripheral blood mononuclear cells in juvenile Idiopathic arthritis: a more relevant cellular biomarker for drug response? *Arthritis Rheum*. 2011;63(Suppl 10):255.
61. Syversen SW, Goll GL, Jorgensen KK, Sandanger O, Sexton J. Effect of therapeutic drug monitoring vs standard therapy during infliximab induction on disease remission in patients with chronic immune-mediated inflammatory diseases: a randomized clinical trial. *Jama*. 2021;325(17):1744-1754. doi:10.1001/jama.2021.4172

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