EXTENDED REPORT

Methotrexate polyglutamates in erythrocytes are associated with lower disease activity in juvenile idiopathic arthritis patients

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ABSTRACT

Objective To determine association of erythrocyte methotrexate polyglutamates (MTX-PG) with disease activity and adverse effects in a prospective juvenile idiopathic arthritis (JIA) cohort.

Methods One hundred and thirteen JIA patients were followed from MTX start until 12 months. Erythrocyte MTX-PGs with 1–5 glutamate residues were measured at 3 months with tandem mass spectrometry. The outcomes were Juvenile Arthritis Disease Activity Score (JADAS)-27 and adverse effects. To determine associations of MTX-PGs with JADAS-27 at 3 months and during 1 year of MTX treatment, linear regression and linear mixed-model analyses were used. To determine associations of MTX-PGs with adverse effects during 1 year of MTX treatment, logistic regression was used. Analyses were corrected for JADAS-27 at baseline and co-medication.

Results Median JADAS-27 decreased from 12.7 (IQR: 7.8–18.2) at baseline to 2.9 (IQR: 0.1–6.5) at 12 months. Higher concentrations of MTX-PG3 (β: −0.006, p=0.005), MTX-PG4 (β: −0.015, p=0.004), MTX-PG5 (β: −0.051, p=0.011) and MTX-PG3–5 (β: −0.004, p=0.003) were associated with lower disease activity at 3 months. Higher concentrations of MTX-PG3 (β: −0.005, p=0.028), MTX-PG4 (β: −0.014, p=0.014), MTX-PG5 (β: −0.049, p=0.023) and MTX-PG3–5 (β: −0.004, p=0.018) were associated with lower disease activity over 1 year. None of the MTX-PGs was associated with adverse effects.

Conclusions In the first prospective study in JIA, long-chain MTX-PGs were associated with lower JADAS-27 at 3 months and during 1 year of MTX treatment. Erythrocyte MTX-PG could be a plausible candidate for therapeutic drug monitoring of MTX in JIA.

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INTRODUCTION

In the treatment of juvenile idiopathic arthritis (JIA), methotrexate (MTX) is the anchor disease-modifying antirheumatic drug, due to its safety and efficacy.[1] However, in 30–40% of JIA patients, MTX is not sufficiently effective.[1–5] Moreover, gastrointestinal adverse effects occur in as many as half of all JIA patients on MTX, potentially leading to decreased MTX efficacy and premature discontinuation of MTX.[6–10] Nevertheless, early effective treatment remains crucial in order to prevent joint destruction and long-term disabilities.[10 11] It is therefore important to provide clinicians with tools that could guide tailor-made treatment decisions early in disease course, for example, to give MTX monotherapy to MTX-responsive patients, or to adjust the MTX dose or give biologicals (in combination with MTX) to those poorly responsive or having adverse effects.[12] To date, we and others identified clinical[13–15] and genetic[16–21] factors associated with MTX efficacy, and constructed a clinical-genetic model for MTX non-response,[12] which could assist clinicians in making individualised treatment decisions.

Besides the aforementioned, measurement of MTX concentrations in blood, the so-called therapeutic drug monitoring (TDM) of MTX, could be a powerful tool in steering tailor-made therapeutic decisions directly. TDM of plasma MTX concentrations is not possible, as MTX in plasma is eliminated within 24 h,[22] and is not correlated with disease activity.[23] However, intracellularly retained MTX could be a reliable TDM tool. MTX, which contains one glutamate residue, is polyglutamated with up to four glutamate chains (MTX-PG1–5)[24] intracellularly, which prevents MTX's efflux by various transporters. MTX-PGs in erythrocytes are representative of polyglutamation in bone marrow progenitors[25] and could therefore be representative of MTX-PG levels in other cell types such as lymphocytes.[26] Polyglutamation enhances MTX's affinity for target enzymes in the folate, purine and pyrimidine pathways,[27] thus promoting MTX's anti-inflammatory effects. Therefore, MTX-PGs could be biomarkers of response to MTX and could thus be used as a TDM tool.

Several groups investigated the association of erythrocyte MTX-PGs with disease activity in rheumatoid arthritis (RA) and JIA. The results were conflicting, showing association of MTX-PGs with lower, but also with higher disease activity,[22 28–34] and no association with disease activity.[17 35] The majority of these studies[17 28–30 32 35] faced two drawbacks which could have influenced their conclusions. First, patients were not prospectively followed from MTX start, which makes a fair comparison of disease activity status between patients difficult. Second, patients used MTX from few months to 22 years, which, given that MTX-PG accumulation is a function of time,[23] complicates comparison of MTX-PG concentrations between patients. Moreover, MTX-PGs have to be measured early after MTX start, if they are to be used as biomarkers of patient's response to MTX and as a TDM tool.

The aim of this study was to determine whether erythrocyte MTX-PGs, measured at 3 months after MTX start, were associated with disease activity and adverse effects in a large prospective JIA cohort, followed for 1 year after MTX start.

PATIENTS AND METHODS

Study design and patients

A prospective investigator-initiated clinical trial on efficacy and adverse effects of MTX (ISRCTN13524271) was performed at University Medical Center, Utrecht, and Erasmus University Medical Center, Rotterdam, The Netherlands, between August 2007 and February 2013. It was approved by the ethics committees of the participating centres, and conducted according to good clinical practice guidelines.

Patients aged 2–18 years, with a confirmed JIA diagnosis[36], starting MTX for treatment of arthritis (not uveitis) without concomitant biological treatment, were included. Those who had stopped MTX for more than 6 months, but restarted MTX due to a relapse, were also included. At MTX start and 3, 6 and 12 months after MTX start, patients' clinical data (table 1) were documented. At the 3-month visit, patients provided a blood sample for MTX-PG quantification. This time-point was chosen because: (A) this is the first occasion for clinicians to evaluate MTX response and make subsequent therapeutic decisions after start; (B) detection of MTX-PGs was expected, given rapid accumulation (7 or 10–20 weeks) of high MTX-PG concentrations.[23 37]

Disease activity

Disease activity, as primary outcome, was assessed during the 12-month follow-up with the composite Juvenile Arthritis Disease Activity Score (JADAS)-27, measured in 27 joints (range 0–57 points).[38]

Adverse effects

Adverse effects (MTX intolerance, hepatotoxicity and bone marrow suppression) were assessed as secondary outcomes during 12 months. MTX intolerance prevalence was determined using the validated MTX Intolerance Severity Score (MISS).[6] It included abdominal pain, nausea and vomiting occurring after, as well as before (anticipatory), and when thinking (associative) MTX administration, accompanied by behavioural symptoms (restlessness, irritability, crying and refusal of MTX).[6] MTX intolerance was defined as a score of ≥6 on the MISS, including at least one anticipatory, associative, or behavioural symptom.[6] Hepatotoxicity was defined as increase in liver enzymes (ALAT and/or ASAT), two times the upper limit of normal, and bone marrow suppression as lymphocyte count $<0.9\times10^9$/L, granulocyte count $<1.5\times10^9$/L and/or thrombocyte count $<20\times10^9$/L.[39]

Quantification of MTX-PGs in erythrocytes

To determine MTX-PG concentrations, EDTA whole-blood samples on ice were obtained at the median of 3 months (range 1.5–4.5 months) after MTX start, and were centrifuged at 1400 g for 10 min to pellet the erythrocytes. The pellets were stored at −80°C until used. MTX-PG1–5 (nmol/L of packed erythrocytes), were measured separately with a novel liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) method, which uses stable isotopes for quantification.[24] Individually measured MTX-PGs were summed up to obtain the total MTX-PG concentration.

Table 1 Baseline characteristics of 113 patients

Female, n (%)	68 (60.2)
Age at MTX start, years, median (IQR)	12.1 (7.5–14.5)
Age at onset, years, median (IQR)	8.8 (3.8–12.3)
JIA subtype, n (%)	
Persistent oligoarticular	26 (23.0)
Extended oligoarticular	16 (14.2)
Polyarticular*	49 (43.4)
Psoriatic	10 (8.8)
Enthesitis-related	10 (8.8)
Systemic onset	2 (1.8)
Core-set criteria, median (IQR)	
Physician global assessment disease activity (0–10)	3 (2.0–4.0)
Joints with limited range of motion	2 (1–5)
Joints with active arthritis	4 (1–9)
CHAQ disability (0–3)	0.9 (0.4–1.6)
Parent/patient global assessment of well being (0–10)†	4.1 (2.0–7.1)
Parent/patient global assessment of pain (0–10)‡	3.9 (1.6–7.0)
ESR (mm/hour)	15.0 (7.0–40.0)
Medication	
Methotrexate dose, mg/m^2/wk, median (IQR)§	9.9 (9.0–11.4)
Folic acid, n (%)¶	113 (100)
NSAIDs, n (%)**	91 (80.5)

*Rheumatoid factor (RF) positive n=8 (16.3% of all polyarticular JIA patients).
†Available in 111 patients.
‡Available in 112 patients.
§Parenteral MTX (n=2 (1.8%)), concomitant treatment with sulfasalazine (n=4 (3.5%)), oral steroids (n=4 (3.5%)), local steroids (n=11 (9.7%)).
¶Doses: 5 mg/week, 24 h after MTX intake.
**Ibuprofen (n=36), naproxen (n=22), indomethacin (n=17), diclofenac (n=14), etoricoxib (n=1), meloxicam (n=1).
CHAQ, Childhood Health Assessment Questionnaire; ESR, erythrocyte sedimentation rate; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; MTX, methotrexate; NSAIDs, Non-steroidal anti-inflammatory drugs.

Statistical analysis

Prior to determining associations of MTX-PGs with JADAS-27 using linear regression analysis, its assumptions pertaining to the outcome (JADAS-27) were checked. Normality of residuals was checked with Normal Probability Plot for JADAS-27 standardised residuals, and homogeneity of variance and linearity of the model were checked by plotting JADAS-27 standardised residuals on the Y axis against predicted standardised values on the X axis. Since linear regression assumptions were violated, JADAS-27 was logarithmically transformed using the common logarithm (log10).

First, multivariate linear regression analysis was performed to determine associations of MTX-PGs, measured at 3 months, with the 3-month JADAS-27 (cross-sectional analysis). The analyses included covariates, potentially affecting disease activity at 3 months: baseline JADAS-27, JIA subtype and baseline non-steroidal anti-inflammatory drug (NSAID) use. Second, analysis of covariance (ANCOVA) was performed to compare the 3-month JADAS-27 (geometric mean) between three groups (tertiles) of MTX-PG concentrations, while correcting for baseline JADAS-27. The 1st tertile reflected the lowest MTX-PG concentrations, whereas the 3rd tertile reflected the highest MTX-PG concentrations. The Bonferroni adjustment was applied for multiple comparisons.

Finally, linear mixed-model analysis was performed to determine associations of MTX-PGs with JADAS-27 during the entire follow-up (longitudinal analysis). This model enables a repeated measurement analysis with unequal periods of time between the visits. Moreover, this model considers each patient to have his own pattern of JADAS-27 over time.

To determine associations of MTX-PGs with adverse effects in the first year of MTX use, multivariate logistic regression was performed, including NSAID use (potentially affecting adverse effect occurrence) as a covariate.

Statistical analyses were carried out with SPSS V.20.0.0 (SPSS, Chicago, Illinois, USA). All comparisons were twosided at α=5%. Linear regression and mixed model were represented as regression coefficients (β), and logistic regression as OR, all with 95% CI.

RESULTS

Patients and disease activity

Of 161 eligible patients, 48 patients were excluded due to a missing blood sample (n=29) and a blood sample at 6 instead of 3 months after MTX start (n=19), resulting in 113 patients included in the analyses (table 1). The starting median MTX dose was 9.9 mg/m^2/week (IQR: 9.0–11.4) (table 1). The median dose was 10.0 (9.1–11.5) at 3 months, 10.5 (9.2–12.7) at 6 months, and 10.8 mg/m^2/week (9.4–13.0) at 12 months upon MTX start. After 3 months of MTX use, 7 (6.2%) of 113 patients discontinued MTX due to insufficient effect (n=4), gastrointestinal intolerance (n=2) or toxicity (elevated liver enzymes: n=1) (table 1). Seven patients (6.2%) received additional medication (hydroxychloroquine (n=1) and anti-TNFα (n=6)). Median JADAS-27 decreased from 12.7 points (IQR: 7.8–18.2) at MTX start to 2.9 points (IQR: 0.1–6.5) after 12 months (table 2).

MTX-PG concentrations in erythrocytes

Concentrations of short-chain polyglutamates MTX-PG1 and MTX-PG2, long-chain polyglutamates MTX-PG3, MTX-PG4, MTX-PG5 and total MTX-PG are shown in table 3. After 3 months of MTX use, the concentration of MTX-PG1, the native form of MTX, was the highest with the median of 25.3 nmol/L, followed by MTX-PG3 (23.0) and MTX-PG2 (18.7). Concentrations of MTX-PGs with 4 and 5 glutamate residues were considerably lower, with medians of 4.2 and 0.7 nmol/L, respectively. The predominant MTX-PGs were MTX-PG1, which accounted for 32.9% and MTX-PG3, which accounted for 32.7% of total MTX-PG.

Long-chain MTX-PGs are associated with lower disease activity 3 months after MTX start

In cross-sectional analysis, higher concentrations of long-chain MTX-PG3, MTX-PG4, MTX-PG5 and their sum MTX-PG3–5 at 3 months were associated with lower JADAS-27 and hence lower disease activity at 3 months after MTX start (table 4). Moreover, these MTX-PGs were also associated with improvement in JADAS-27 between 3 months and the baseline (MTX-PG3: β=0.006 (−0.010 to −0.001), p=0.010; MTX-PG4: β=−0.015 (−0.025 to −0.005), p=0.005; MTX-PG5: β=−0.048 (−0.087 to −0.009), p=0.017 and MTX-PG3–5: β=−0.004 (−0.007 to −0.001), p=0.006). Conversely, short-chain MTX-PG1, MTX-PG2 and total MTX-PG were not significantly associated with JADAS-27 (table 4) and JADAS-27 improvement (data not shown).

The inverse relationship between MTX-PGs and JADAS-27 is also shown in figure 1. JADAS-27 showed the same pattern for all long-chain MTX-PGs and total MTX-PG, notably the higher the MTX-PG concentrations, the lower the JADAS-27. This pattern was not observed for JADAS-27 corresponding to short-chain MTX-PGs. The JADAS-27 geometric means (95% CI) corresponding to the 3rd MTX-PG tertile (highest concentration) were significantly lower than those of the 1st tertile for: MTX-PG3 (4.9 (3.9 to 6.2) vs 8.6 (6.8 to 11.0), p=0.004); MTX-PG4 (5.3 (4.1 to 6.7) vs 8.2 (6.4 to 10.4), p=0.037), MTX-PG5 (5.1 (4.0 to 6.6) vs 8.3 (6.5 to 10.7), p=0.021), MTX-PG3–5 (5.1 (4.0 to 6.5) vs 8.8 (6.9 to 11.2), p=0.006), and total MTX-PG (5.1 (4.0 to 6.6) vs 7.9 (6.2 to 10.2), p=0.042) (figure 1).

Additionally, compared to patients who continued MTX, long-chain MTX-PG concentrations (median, IQR) were significantly lower in patients who stopped MTX due to insufficient response (n=4) (MTX-PG3: 7.6 (1.8 to 14.3) vs 23.3 (14.9 to

Table 2 JADAS-27

Time point	Median (IQR)
MTX start	12.7 (7.8–18.2)*
3 months	5.9 (2.8–11.5)†
6 months	4.9 (1.4–9.4)‡
12 months	2.9 (0.1–6.5)§

*Determined in: 111 patients.
†Determined in: 110 patients.
‡Determined in: 104 patients.
§Determined in: 101 patients.
JADAS, Juvenile Arthritis Disease Activity Score; MTX, methotrexate.

Table 3 Concentrations of MTX-PGs and proportion of individual MTX-PGs in relation to total MTX-PG

	Concentration, nmol/L* median (IQR)	Proportion, % median (IQR)
MTX-PG1	25.3 (17.9–33.8)	32.9 (25.5–44.4)
MTX-PG2	18.7 (14.8–24.3)	25.7 (21.5–29.5)
MTX-PG3	23.0 (13.9–33.9)	32.7 (24.0–38.5)
MTX-PG4	4.2 (2.0–8.7)	6.4 (3.6–9.5)
MTX-PG5	0.7 (0.3–1.8)	0.9 (1.5–0.9)
Total MTX-PG	79.0 (53.4–103.2)	Reference

*Expressed as nmol/L of packed erythrocytes.
MTX-PG, methotrexate polyglutamate.

Table 4 Associations of MTX-PGs with JADAS-27

	Cross-sectional analysis* (β (95% CI))	Longitudinal analysis† (β (95% CI))
MTX-PG1	0.000 (−0.004 to 0.003)	0.001 (−0.003 to 0.005)
MTX-PG2	−0.002 (−0.010 to 0.006)	−0.001 (−0.007 to 0.011)
MTX-PG3	−0.006 (−0.010 to −0.002)‡	−0.005 (−0.010 to −0.001)§
MTX-PG4	−0.015 (−0.026 to −0.005)‡	−0.014 (−0.026 to −0.003)§
MTX-PG5	−0.051 (−0.090 to −0.012)‡	−0.049 (−0.092 to −0.007)§
MTX-PG3–5	−0.004 (−0.007 to −0.001)‡	−0.004 (−0.007 to −0.001)§
Total MTX-PG	−0.002 (−0.003 to 0.000)	−0.001 (−0.003 to 0.001)

*Cross-sectional: multivariate linear regression for association of MTX-PGs with JADAS-27 at 3 months.
†Longitudinal: linear mixed model for association of MTX-PGs with JADAS-27 during the first year of MTX treatment.
‡Significant p values (<0.05) specified from top to bottom: p=0.005, p=0.004, p=0.011, p=0.003.
§Significant p values (<0.05) specified from top to bottom: p=0.028, p=0.014, p=0.023, p=0.018.
Example: The significant associations of MTX-PGs with JADAS-27 represent the following: with 1 nM increase in MTX-PG, the logarithmic JADAS-27 decreases by the respective β.
JADAS, Juvenile Arthritis Disease Activity Score; MTX-PG, methotrexate polyglutamate.

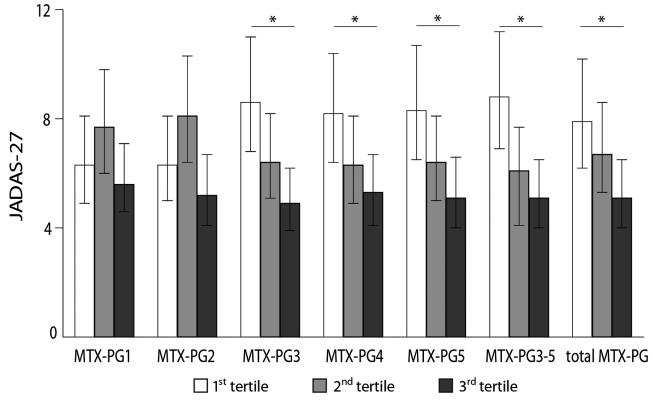

Figure 1 Juvenile Arthritis Disease Activity Score (JADAS)-27 at 3 months corresponding to methotrexate polyglutamates (MTX-PGs) divided into tertiles. Bars show geometric means (back-transformed logarithmic JADAS-27) and 95% CIs. The 1st tertile represents the lowest and the 3rd tertile the highest MTX-PG concentrations. MTX-PG concentrations (nmol/L) within tertiles are: MTX-PG1—1st: lowest to 20.8, 2nd: 20.9 to 29.5, 3rd: 29.6 to highest; MTX-PG2—1st: lowest to 15.6, 2nd: 15.7 to 22.0, 3rd: 22.1 to highest; MTX-PG3—1st: lowest to 17.2, 2nd: 17.3 to 29.4, 3rd: 29.5 to highest; MTXPG4—1st: lowest to 2.8, 2nd: 2.9 to 6.5, 3rd: 6.6 to highest; MTX-PG5—1st: lowest to 0.3, 2nd: 0.4 to 1.1, 3rd: 1.2 to highest; MTX-PG3–5—1st: lowest to 20.6, 2nd: 20.7 to 37.1, 3rd: 37.2 to highest; total MTX-PG—1st: lowest to 61.7, 2nd: 61.8 to 89.5, 3rd: 89.6 to highest concentration. The higher the concentrations of long-chain MTX-PGs and total MTX-PG, the lower the JADAS-27. This pattern was not observed for short-chain MTX-PGs. JADAS-27 corresponding to the 3rd MTX-PG tertile was significantly lower for long-chain MTX-PGs, their sum and total MTX-PG ($p<0.05$). JADAS: Juvenile Arthritis Disease Activity Score; MTX-PGs: methotrexate polyglutamates.

34.3), p=0.009; MTX-PG4: 0.8 (0.1 to 3.1) vs 4.6 (2.1 to 8.8), p=0.020; MTX-PG5: 0.2 (0.1 to 0.7) vs 0.7 (0.3 to 1.8), p=0.046). In this group, MTX dose at start (median, IQR) and during follow-up (data not shown) were also significantly lower (6.3 (5.3 to 7.8) vs 9.9 (9.1 to 11.5)). Conversely, MTX-PG levels and MTX dose (data not shown) were comparable between patients who received concomitant medication due to insufficient response to MTX (n=7) and those who received MTX monotherapy (MTX-PG3: 32.4 (21.0 to 35.8) vs 23.7 (13.5 to 33.4), p=0.165; MTX-PG4: 6.8 (3.5 to 8.6) vs 4.0 (1.9 to 8.4), p=0.278; MTX-PG5: 1.0 (0.6 to 1.8) vs 0.7 (0.3 to 1.7), p=0.345).

Long-chain MTX-PGs are associated with lower disease activity in the first year of MTX treatment

In the longitudinal analysis, higher concentrations of long-chain MTX-PG3, MTX-PG4, MTX-PG5 and MTX-PG3–5 at 3 months were significantly associated with lower JADAS-27 over time, and hence with lower disease activity in the first year after MTX start (table 2). The effect of abovementioned MTX-PGs on disease activity did not change over time ($p>0.05$).

MTX-PGs are not associated with adverse effects

MTX intolerance prevalence was determined in 89 patients in the first year after MTX start, of whom 45 (50.6%) were intolerant. In the first year after MTX start, hepatotoxicity was observed in 6 (5.3%) of 113, and bone marrow suppression (lymphopenia and granulopenia) in 4 (3.5%) of 112 patients.

MTX-PGs, measured at 3 months, were not associated with MTX intolerance (results shown for total MTX-PG) (OR: 0.99 (1.00 to 1.01), p=0.72), hepatotoxicity (OR: 1.02 (1.00 to 1.04), p=0.08) or bone marrow suppression (OR: 0.98 (0.90 to 1.10), p=0.57) in the first year after MTX start.

DISCUSSION

In a prospective JIA cohort, long-chain MTX-PG3, MTX-PG4, MTX-PG5 and MTX-PG3–5, measured after 3 months of MTX use, were associated with lower JADAS-27 at 3 months. Long-chain MTX-PGs were also associated with lower JADAS-27 during 1 year of MTX treatment. MTX-PGs were not associated with adverse effects.

In line with our findings, higher concentrations of total MTX-PG,[22 31 32] long-chain MTX-PG3[29 37] and MTX-PG5[34] have been associated with response to MTX in RA patients. Conversely, in a single study in RA, MTX-PGs were not associated with lower disease activity.[30] In fact, MTX-PG5 was associated with high rather than low disease activity.[30] In JIA, two studies also failed to show associations of total and individual MTX-PGs with inactive disease and response to MTX.[17 35] In these cross-sectional studies, contrary to our study, disease status was determined retrospectively, which could impact the reliability of the evaluated disease activity status and impede a fair comparison of disease activity status between patients. Furthermore, included patients used MTX for highly variable periods of time, which makes the comparison of MTX-PG concentrations between patients difficult, given that MTX-PG accumulation is dependent on the time of exposure to MTX.[23 37] These issues could have influenced their conclusions on the association of MTX-PGs and disease activity.

Our finding that long-chain MTX-PGs are associated with lower disease activity is consistent with the notion that longer-chain MTX-PGs are more potent inhibitors of target enzymes in the folate, purine and pyrimidine pathways[27] and in turn more potent mediators of MTX's therapeutic efficacy. Nevertheless, a recent study in RA showed that short-chain MTX-PG2 was associated with disease activity score (DAS)28 improvement after 16 weeks of MTX use.[33] In our validated study in RA, short-chain MTX-PG2 and long-chain MTX-PG3 and MTX-PG4, measured longitudinally, were associated with the lower DAS28 during 9 months of MTX treatment.[40]

By contrast with MTX-PG associations with disease activity, no MTX-PGs were associated with adverse effects in our cohort. However, another recent study in JIA did show associations of MTX-PG3–5 with gastrointestinal symptoms and elevated liver enzymes.[17]

In the present study, relationship between MTX-PG and adverse effects could have been attenuated by the standard-of-care folic acid use, as this supplement reduces the occurrence of MTX-related adverse effects.[41 42] Moreover, low prevalence of hepatotoxicity (5.3%) and bone marrow suppression (3.5%) in our cohort could have led to spurious conclusions on MTX-PG associations with these adverse effects. In line with our findings in JIA, no associations of MTX-PGs with adverse effects were found in RA.[28–30 40] Intracellular folate status, rather than MTX-PGs, could be associated with adverse effects. Lower erythrocyte folate polyglutamate concentrations were associated with a history of MTX toxicity in juvenile arthritis patients not currently receiving MTX.[43] Conversely, in our validated study in RA, baseline erythrocyte folate concentrations were not associated with adverse effects.[44]

Similar to earlier studies, long-chain MTX-PG3 was the predominant polyglutamate sybtype.[30 37 45 46] However, MTX-PG concentrations found in our cohort, were lower than previously reported.[30 37 45 46] This could be explained by differing

methods used to measure MTX-PGs. We employed MS using stable isotopes to quantify MTX-PGs,[24] whereas others used either MS without stable isotopes[46] or liquid chromatography without MS.[30 37] These methods might be less specific and prone to interferences by compounds similar to MTX, such as folates.[24] Nevertheless, compared with our RA study,[40] where MTX-PGs were measured using the same MS method, MTX-PG levels in JIA remained lower, likely due to lower age.[37 45 46] Compared with previous findings in JIA,[46] short-chain MTX-PG concentrations were higher (MTX-PG1: 25.3 vs 16.8; MTX-PG2: 18.7 vs 11.8) and long-chain MTX-PG concentrations lower in our cohort (MTX-PG3: 23.0 vs 37.1; MTX-PG4: 4.2 vs 10.3; MTX-PG5: 0.7 vs 2.7). This could be explained by longer MTX use in the abovementioned study (median: 3 years) compared with an average of 3 months in our study, since longer exposure to MTX leads to selective enrichment of long-chain MTX-PGs, at the expense of short-chain MTX-PGs.[37]

The present study is the first step towards the use of MTX-PGs as a TDM tool, as it showed that long-chain MTX-PGs, measured early after MTX start, are related to low disease activity 3 months after MTX start, and also during the first year of MTX treatment. MTX-PGs could be used as a TDM tool to guide clinical decision making, notably to determine whether a patient would benefit from an increase in MTX dose, or whether additional medication, such as biologicals, should be given. In the present study, potential use of MTX-PGs as a TDM tool can be illustrated in patients who stopped MTX and those who received additional medication, due to insufficient effect. Patients who discontinued MTX received lower MTX doses and had lower long-chain MTX-PG concentrations than those who continued MTX. Instead of stopping MTX, these patients may have benefited from MTX dose escalation. On the other hand, patients on additional medication at 6 months had similar MTX doses and MTX-PG concentrations at 3 months, as patients on MTX monotherapy. They remained non-responders, in spite of optimal MTX treatment (reflected by adequate polyglutamation). If timely monitored with TDM, they could have received additional medication earlier than 6 months after MTX start. Taken together, TDM of MTX-PGs could guide clinicians to escalate MTX dose in patients with a low polyglutamation rate, and to offer biologicals in non-responders with adequate polyglutamation.

In order to use MTX-PGs as a TDM tool, MTX-PG pharmacokinetics, in response to MTX dose escalation and/or changes in the route of administration, needs to be determined with sequential MTX-PG measurements during the first year of MTX treatment. Indeed, higher concentrations of preferentially long-chain MTX-PGs accumulate in patients using higher MTX dose and receiving parenteral MTX.[37 45 46] In our study, higher concentrations of long-chain MTX-PG3,4 and total MTX-PG were associated with higher baseline MTX dose. Knowing how to influence accumulation and concentrations of MTX-PGs, with the aim of maximising response to MTX, could enable optimisation of MTX treatment for individual patients. Pharmacokinetics will therefore be the focus of future research.

In conclusion, this is the first study to show that higher concentrations of long-chain MTX-PG3, MTX-PG4 and MTX-PG5 at 3 months of MTX use are associated with lower disease activity at 3 months and during 1 year of MTX treatment in a prospective JIA cohort. MTX-PGs were, however, not associated with adverse effects. Long-chain MTX-PGs, measured early after MTX start, are a potential TDM tool in JIA.

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Methotrexate polyglutamates in erythrocytes are associated with lower disease activity in juvenile idiopathic arthritis patients

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