Prediction of response and adverse events to methotrexate treatment in patients with rheumatoid arthritis

Methotrexate is the disease-modifying antirheumatic drug of first choice for most patients with rheumatoid arthritis. Although methotrexate has been on the market for a few decades now, we are still unable to predict with great accuracy who will respond to methotrexate treatment and who will develop adverse events. A number of studies have identified several demographic, clinical and genetic factors associated with (non)-response or adverse events, but results are controversial. This paper describes the findings to date and possible explanations for the inconsistent findings.

KEYWORDS: adverse events ▪ clinical ▪ demographic ▪ genetic ▪ liver enzymes ▪ methotrexate ▪ predictors ▪ response ▪ rheumatoid arthritis

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Half a century ago, methotrexate (MTX) was introduced as a therapy for cancer and since approximately 1980 it has been used to treat patients with rheumatoid arthritis (RA). Since the early 1990s, there has been a gradual increase in the use of MTX in patients with RA [1,2]. Data from Finland shows that of those patients recruited to the Jyvaskyla cohort between 1988 and 1989, 20% used MTX 5 years after registration, which increased to 70% in those patients recruited between 1996 and 1997 [3]. A similar trend was observed in the Norfolk Arthritis Register (UK) including patients with inflammatory polyarthritis, in which these percentages were respectively 20.3% in the 1990–1994 cohort and 55.2% in 2000–2004 cohort [4]. These trends are a reflection of the fact that MTX is now the disease-modifying antirheumatic drug (DMARD) of first choice in the treatment of most patients with RA [5,6].

Compared to many other (conventional) DMARDs, MTX is the most effective and is better tolerated for a longer period of time [7]. Treatment discontinuation due to toxicity occurs in approximately 10–37%, with the most common adverse events (AEs) being gastrointestinal events and elevated liver enzymes [8,9]. MTX is relatively cheap, especially compared with biologic drugs, and, therefore, in many countries, guidelines for RA treatment and prescription of biologic drugs recommend MTX as the first DMARD as monotherapy or in combination with other conventional DMARDs or steroids [101].

It is particularly important to be able to identify those patients who do well on MTX, because these patients could then be treated with an effective and relatively cheap medication. It is, however, equally important to identify those patients who do not respond to MTX treatment, because these patients could then be fast-tracked to biologic agents in order to protect their joints from progressive damage resulting in long-term disability and decreased quality of life.

In this paper, we will discuss demographic, clinical and genetic predictors of MTX (non)-response and AEs, with a focus on liver toxicity, in patients with RA treated with MTX.

Pharmacokinetics of MTX

The actual mechanisms of action of low-to-moderate-dose MTX (7.5–30 mg/week), the dose range mostly applied for RA, are still not fully understood, but it is thought that the anti-inflammatory effects, mediated by adenosine release, are more important than the antiproliferative effects [10–12]. MTX is internalized by the reduced folate carrier (SLC19A/RFC) to enter the cell, and impaired transport is correlated with MTX resistance. This is mainly observed in cancer treatment where higher doses of MTX are prescribed than for treatment of RA [13,14]. Once internalized, MTX requires intracellular polyglutamation, and it is controlled by the polyglutumation–deconjugation cycle that is instigated by the enzymes FPGS and GGH, respectively [15]. It is these active MTX polyglutamates that determine MTX functional status and have an important role in directly suppressing various enzymes, such as DHFR, TYMS, AICAR and ATIC, as well as having an indirect effect on MTHFR (Figure 1) [15–17] . Although most studies to date have focused on RFC as the only route by which MTX enters the cell, it is thought that

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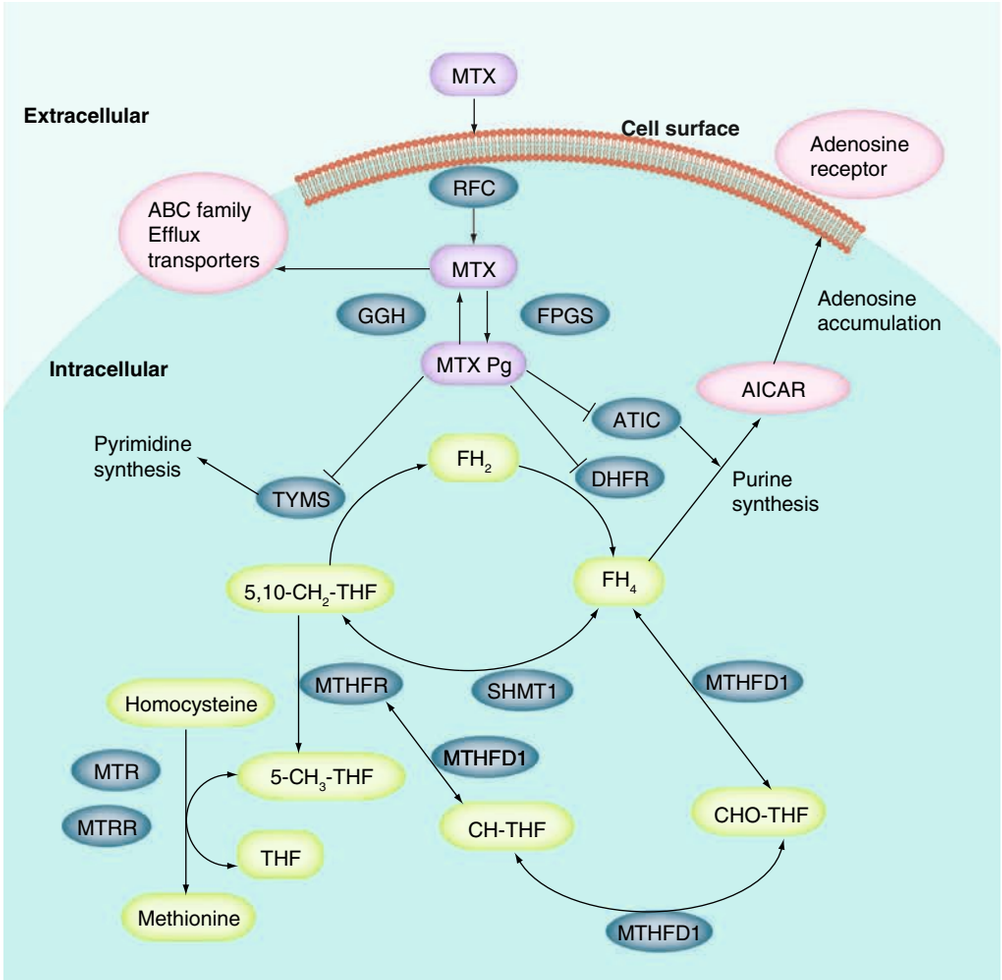

Figure 1. The methotrexate metabolic pathway. To enter the cell, MTX is internalized by the reduced folate carrier (SLC19A/RFC). Once internalized, MTX is controlled by the polyglutumation–deconjugation cycle that is instigated by FPGS and GGH, respectively. It is these active MTX polyglutamates that determine MTX functional status and have an important role in directly suppressing various enzymes, such as DHFR, TYMS, AICAR and ATIC, as well as having an indirect effect on MTHFR.
FPGS: Folylpolyglutamyl synthetase; GGH: γ-glutamyl hydrolase; MTHFD1: Methylenetetrahydofolate reductase; MTHFR: Methylenetetrahydofolate reductase; MTR: Methionine synthase; MTRR: Methionine synthase reductase; MTX: Methotrexate; MTX-Pg: MTX polyglutamate; RFC: Reduced folate carrier; SHMT1: Serine hydroxymethyl transferase.
Adapted from [17].

folate receptor-β, which is expressed in particular on synovial macrophages, might also be a transport protein for MTX in arthritic joints [18].

Factors associated with MTX (non)-response

Demographic & clinical factors

Since RA is increasingly being diagnosed in people aged over 60 years, and MTX is cleared by the kidney, there might be a change in response to MTX with increasing age since there is an age-related decline in renal function. When pooling data from 11 clinical trials containing 496 patients treated with MTX, age did not seem to affect response to MTX treatment [19]. However, in a more recent paper, including patients from the SWEFOT study, older patients were more likely to respond to MTX 3–4 months after starting to take MTX [20]. The role of renal function is more controversial, with conflicting findings reported in the literature [19,21]. One of the reasons for these conflicting results could be the measurement of renal function. Some studies measured serum creatinine, which is dependent on renal function, muscle mass and age, and may not accurately reflect glomerular filtration rate, which has been used in other studies. Glomerular filtration rate can be

measured directly by using 24-h urinary assessment, which may not always be logistically feasible to measure, or estimated using a formulae including age, weight and serum creatinine.

Other demographic and clinical factors include (premenopausal) female patients being less likely to respond to MTX treatment than men in some studies [20–22], but not all [23]. In early RA, a strong association between smoking and a decreased likelihood of MTX response was observed in the SWEFOT study [20], but not in itself in the MTX monotherapy arm of the BeSt study [22]. Rheumatoid factor positivity and anticyclic citrullinated peptide antibody positivity [20,22,23] have not been associated with response in some studies. Conversely, in the PROMPT trial of patients with early undifferentiated arthritis, the only patients who derived benefit from treatment with MTX were those who were anticyclic citrullinated peptide antibody positive [24]. In one study, smoking in combination with rheumatoid factor positivity predicted poor response [22]. These results are interesting since both seropositivity and smoking are related to worse disease progression, and one might expect that these factors are therefore also associated with treatment response.

A few studies looked at the association between functional disability and disease activity measured at baseline with low disease activity or remission later on. The Health Assessment Questionnaire (HAQ) score of responders was lower compared with nonresponders in one study [20,22], but no association was found in another study [23]. Some studies examining disease activity as predictors of response (e.g., European League against Rheumatism response, American College of Rheumatology [ACR] response, low Simplified Disease Activity Index [SDAI] or Clinical Disease Activity Index [CDAI] score) suggest that patients with low disease activity are more likely to respond [20,21,25], but others find no such association with response (inefficacy according to physician) [23]. This may be due to the difference in outcome measure between the studies. The independent variable and the outcome measure of the first three studies are based on the same measurement, whereas in the latter study another outcome measure was used.

NSAIDs are often coprescribed with MTX therapy, which may cause a pharmacokinetic interaction and a subsequent increase in blood MTX concentration due to a decrease in glomerular filtration of MTX by NSAIDs via reduction of renal blood flow with inhibition of prostaglandin synthesis, inhibition of MTX tubular secretion and competition for protein-binding sites. Since MTX and most NSAIDs are mainly excreted into urine, it is expected that the competition between renal transporters for MTX and NSAIDs such as OAT3 play an important role in this interaction [26]. Uptake of MTX by hOAT3 cells is inhibited by most NSAIDs in a concentration-dependent manner, but not aspirin, salicylate, tiaramide and acetaminophen [26]. Not many studies have investigated the influence of NSAIDs on response or AEs in patients receiving MTX. In one study, patients using NSAIDs were more likely to respond than those who did not use NSAIDs, although it is not known which NSAIDs these patients used [21].

Genetic factors

With any particular drug, a range of genetic factors and biochemical changes related to its mechanism of uptake, retention, target cellular action and ultimate disposal will influence drug responses. In some studies, patients who were shared epitope positive are less likely to respond to MTX monotherapy than patients who were shared epitope negative [27,23]. However, no association was found in another study [28]. Aside from genes already known to play a role in susceptibility to RA disease, polymorphisms in genes encoding key enzymes in the MTX metabolic pathway and receptors have been investigated with conflicting results. The most commonly studied gene is *MTHFR*, with most studies including two specific SNPs – 1298A/C (rs1801133) and 677C/T (rs1801131) – which predict response to or AEs caused by MTX. Both SNPs have been linked with altered phenotypes and adverse drug reactions. Meta-analyses have been published to synthesize the available evidence and the largest of these meta-analyses concluded that there was no association with either polymorphism [29–31].

A number of studies investigated the association between one or more polymorphisms of these metabolic pathway genes and (non)-response to MTX treatment. Again, results were not consistent across studies, but a positive association between SNPs in any of the following genes was observed in one or more studies: *ITPA* [22,32,33], *ATIC* [17,22,33–39], *DHFR* [40], *GGH* [17,41,42], *AMPD1* [22,33,37], *MTHFD1* [22,43], *SLC19A1* [17,34,36,38,40,44–50], *FPGS* [37,41,44], *TYMS* [34–36,49,51–53] and *SHMT1* [35,39,49]. In a recent study, SNPs in these ten pathway genes were tested in a relatively large cohort (n = 309), especially compared with other studies, of patients with RA treated with MTX. In this study, in which data on efficacy were obtained retrospectively, four SNPs in the *ATIC* gene, six SNPs in the *SLC19A1* gene region and one single

SNP in the *GGH* gene region were associated with efficacy [17].

MTX polyglutamates

Prior to being taken up by the cell, conversion to 7-OH-MTX reduces the ability for uptake by the RFC. MTX contains one glutamate moiety and it is referred to as $MTXGlu_1$. $MTXGlu_1$ and the products of intracellular glutamation ($MTXGlu_2$, $MTXGlu_3$, $MTXGlu_4$ and $MTXGlu_5$) are collectively referred to as $MTXGlu_n$. It is these intracellular MTX polyglutamates (PGs) that allow MTX to remain within the cell and mediate the downstream effects of the drug. The ability to increase and decrease the number of glutamates on MTX is under enzymatic control and will ultimately be the result of the amount of MTX absorbed from the GI tract and taken up into the cell, and the balance of poly- and de-glutamation enzyme activity. In a cross-sectional study, lower red blood cell MTX PG levels (PG_3) were associated with a higher number of tender and swollen joints, higher disease activity and higher mHAQ [36]. In another cross-sectional study, no association between $MTXGlu_n$ and reduced disease activity was observed, and, controversially, a significant positive association between $MTXGlu_5$ and high disease activity was observed after correction for MTX dose and other possible confounding factors such as older age, lower estimated GFR and smoking [54]. In the latter study, a positive association between red blood cell folate and disease activity was, however, observed. Ingested folates are also transported into cells by RFC and folate receptors and compete with MTX as substrate, for polyglutamatin by FPGS, and high concentrations of intercellular folates result in a decrease in MTX polyglutamation [55,56]. However, more research is necessary into the exact uptake, retention and disposal mechanisms of MTX and folate since, in general, folate intake does not seem to interfere with MTX treatment response [57].

Factors associated with AEs

Clinical & demographic factors

The most common AEs in patients with RA treated with MTX are gastrointestinal events and liver toxicity [8,58]. The focus of this section will be on clinical and demographic factors associated with liver toxicity, especially elevated liver enzymes (ALT or AST). Results from a systematic review show that the incidence rate of elevated liver enzymes in the first 3 years was estimated to be 13 per 100 patient-years, with a cumulative incidence of 31% (i.e., cumulative incidence >49%), upper limit of normal (ULN) and 17% >two- to three-times ULN. In the CAMERA study, comparing an intensive MTX therapy approach (I group) with conventional MTX therapy approach (C group), 42.3% in the I group and 21.4% in the C group had AST >1*ULN and, 47.0% and had 29.0% ALT >*ULN, respectively [58]. In both treatment groups, starting-dose MTX was 7.5 mg/week and maximum-dose MTX 30 mg/week. On average, the dose of MTX for completers was 16.1 mg/week (95% CI: 14.8–17.3) in the I group and 14.0 mg/week (95% CI: 13.1–14.8) in the C group.

The decline of renal function with increased age, the use of coprescribed drugs such as NSAIDs and diuretics in patients with RA, and the change of pharmacokinetics with increasing age, as well as other comorbidities, may contribute in an additive or synergistic way to MTX AEs. Similar to the contrasting findings for predictors of (non)-response, the results of some studies investigating predictors of liver toxicity are also inconclusive. Age [19,21,59,60], gender [61,62], obesity and increased weight [21,61,62], alcohol intake [21], untreated hyperlipidemia [61], the duration of treatment [59], dose and cumulative dose [58,59,62], absence of folate supplementation [21,63], baseline liver enzyme values [58,62], and baseline creatinine at baseline [58] have all been investigated as possible predictors of increased liver enzymes or severe liver disease.

Older age was associated with increased liver enzymes in one study [59], but not in others [19,21,60,62]. Female gender was associated with increased liver enzymes in a small retrospective study [62], whereas men were more likely to have abnormal AST results in another study (univariate regression analysis <0.05; trend in stepwise regression analysis $p = 0.0522$) [61]. However, in the latter study men did use less folate and drank more alcohol. A few studies found an association between obesity and increased liver enzymes [21,62], but others did not [61]. Since in the general population obesity is also associated with increased ALT and AST levels [63], it is not known whether the association found in RA patients treated with MTX is due to an additive effect of obesity on MTX or not. Similarly, hyperlipidemia, a known risk factor for increased liver enzymes in the general population, has been reported as an independent risk factor for increased liver enzymes in MTX-treated RA patients [61]. Alcohol consumption within normal recommended limits may also contribute to changes in liver function tests and in liver cirrhosis, especially in the context of obesity/insulin

resistant states and/or fatty liver. Therefore, patients who are about to start MTX therapy are recommended to reduce their alcohol intake to a minimum. Alcohol was not associated with increased liver enzymes in a 48-week randomized placebo-controlled trial [21] and in a large retrospective cohort study [61], but it is not known if advice about alcohol intake was given to these patients prior to MTX starting.

High MTX dose [62] and high cumulative-dose MTX [59] have been found to be associated with increased liver enzymes. In the CAMERA study, the average MTX dose prior to the first hepatic event was higher in the I group compared with the C group, but the cumulative dose between the two treatment groups was similar [58].

The use of folinic or folic acid in patients treated with MTX has been investigated in a number of studies and reviewed by Whittle *et al.* [57]. MTX inhibits the enzyme dihydrofolate reductase, resulting in depletion of reduced folates. The latter act as donors of 1-carbon moieties in the formation of metabolic intermediates, including purines, deoxythymidylate monophosphate and methionine, which may lead to a state of effective folate deficiency possibly causing increased gastrointestinal events and increased liver enzymes [64]. Although the guidelines regarding prescription of folic or folinic acid in patients with RA treated with MTX varies across countries, folic acid supplementation is likely to reduce the incidence of liver function test abnormalities and may reduce the incidence of gastrointestinal intolerance [57].

Genetic predictors of AEs

Fewer studies have investigated genetic predictors of MTX-related AEs. The majority of studies have reported on the association with the aforementioned *MTHFR* gene and either an increase or reduction in AEs were found. Two meta-analyses reported on AEs associated with this gene, one reporting that the *MTHFR* 677T SNP was associated with AEs, although the more recent meta-analysis suggested that there was no confirmed association [30,31], suggesting that further large studies are required. Other genes that pose interesting targets are the *ATIC* gene, which plays a role in purine synthesis and the adenosine pathway, *ADORA2A*, ABC transporter genes (*ABCC2, ABCB1* and *ABCG2*) and *DHFR*, which have all been reported to be associated with AEs in one or more studies.

However, response to MTX is complex and is likely to be under the control of several genes, and it may be that multigene indices provide a stronger predictive capability. Further to these studies looking at single SNPs in specific genes, various groups have taken the approach of developing composite pharmacogenetic indices to measure both efficacy and toxicity [34–36,40]. Initial indices were based on a composite score of the homozygous variant genotypes and then a later revision has included low penetrance heterozygote variant genotypes, with a more recently described index containing information on clinical variables as part of the model [22]. Indeed the current indices remain to be further validated across larger patient populations to really understand their clinical validity [65].

Discussion

Although MTX has been prescribed as treatment for RA for a few decades now, we are unfortunately still not able to predict with great accuracy who is going to respond to MTX and who will develop MTX-related AEs. In this overview, we reported the results of a number of previously published studies. The results published to date are very controversial – it is not possible to define a core set of independent predictors of response, and another independent core set of predictors of AEs. As to be expected, some factors associated with a good response are also associated with an increased risk for AE. There are, however, several problems when comparing data from different studies, including: methodological issues, use of other DMARDs or steroids in part of the study population, differences in outcome measures, administration of MTX and inclusion of different independent clinical variables, demographic variables and genetic predictors.

There were some methodological issues that may explain part of the controversial findings or lack of findings. Some of the studies may have been underpowered to detect a statistically significant association. In particular, in the genetic studies, the sample sizes were relatively small (<300). Some studies only performed univariate analyses and no adjustments for possible confounding factors were made to determine the independent association of demographic, clinical and genetic factors with (non)-response or AEs.

Most of the studies discussed in this manuscript included patients treated with MTX as monotherapy. However, in some observational studies the use of steroids and other DMARDs were allowed and in these studies a subpopulation of the participating patients may have used these drugs. In some other studies, patients started with MTX monotherapy, but other DMARDs could be added at a later stage. It is, however,

not known whether this may have had an impact on the associations found or not. Definitions for (non)-response and liver toxicity also varied across studies. (Non)-response as an outcome variable was based on the disease activity score (either based on the 28 or 44 joint count), SDAI, CDAI, ACR response and percentage improvement in clinical and laboratory values. In the SWEFOT study, slightly different predictors of response (i.e., for gender) were found when predictors for the primary response outcome (i.e., European League against Rheumatism response) were validated using SDAI, CDAI or ACR20 [20]. For liver toxicity, definitions included AST or ALT >2*ULN, AST or ALT >3*ULN, or serious liver disease confirmed by histology. Although there are suggested guidelines available to monitor liver toxicity [66], no standardized definitions for liver toxicity as an outcome measure are available for cross-sectional studies and clinical trials (e.g., one measurement over time >2–3 ULN, withdrawal due to increased liver enzymes, the number of times liver enzyme values >1–2 ULN divided by the time points liver enzyme values measured).

Some of the controversial findings between studies may also partly be explained by the range in MTX dose prescribed and route of administration of MTX in these studies. There is no linear relationship between MTX route of administration, dose intake and bioavailability of MTX, especially for higher doses [67,68]. High starting-dose MTX or fast dose escalation are associated with better response compared with low starting dose and slow escalation [6,58,69,70], but at an increased risk of toxicity. Response to treatment may also be influenced by the patient's existing beliefs about the likely effectiveness of the drug, and by whether they have actually taken the medication (adherence). It has been shown that only two-thirds of patients are at least 80% adherent with MTX therapy, which resembles adherence to other DMARDs [71]. Poor adherence accounts for significant worsening of the disease, death and increased healthcare costs in other patient populations [72–74]. In a 10-year longitudinal study, MTX compliance was predicted by longer disease duration, low-to-moderate disease activity, and the presence of a diagnosis of ulcer/mild liver disease [75]. However, little information is available about the relationship between adherence and response to MTX treatment in patients with RA. It is therefore important to keep in mind the possible impact adherence might have had in the different studies when interpreting some of the results.

Most studies to date have either focused on identifying clinical and/or demographic risk factors or on genetic risk factors for response to MTX or MTX-related AEs. Combining clinical and genetic data, a pharmacogenetic model was developed to predict efficacy in patients treated with MTX monotherapy [22]. The final model, based on data from 205 patients, consisted of sex, RF, smoking status, the disease activity score and four polymorphisms in the *AMPD1, ATIC, ITPA* and *MTHFD1* genes. This model, however, needs to be validated in another larger cohort.

In conclusion, several demographic, clinical and genetic factors have been identified as predictors of MTX treatment response or MTX-related AEs. However, findings are inconsistent across studies and most factors only explain a small amount of the variation.

Future perspective

The development of a model for demographic, clinical, genetic and possible other factors to predict which patients with RA will respond to MTX treatment will be the ultimate goal for the future. However, more research is necessary to identify other factors, including biomarkers associated with uptake and retention, or patient-related factors, which may be associated with response to MTX but have not yet been investigated in great detail. Such a model could be applied in daily practice before MTX treatment starts, or early in the course of MTX treatment to predict whether individual patients should either start or continue MTX treatment. If the probability of response is high, patients should continue to use MTX, an effective and relatively cheap drug. However, if the probability of response is low, patients should be fast-tracked to biologic agents to prevent long-term disability. A similar model could be developed for the development of AEs. This model could be used for individual patients to determine whether the beneficial effects of MTX treatment may outweigh the expected development of AEs or not. In addition, this information could be used to adapt treatment strategy (e.g., lower dose and route of administration) or change modifiable risk factors (e.g., alcohol intake and weight).

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Executive summary

Methotrexate for treatment of rheumatoid arthritis

- Methotrexate (MTX) is the disease-modifying antirheumatic drug of first choice for most patients with rheumatoid arthritis.

MTX response & adverse events

- Treatment discontinuation due to toxicity occurs in approximately 10–37% of patients; with the most common adverse events (AEs) being gastrointestinal events and elevated liver enzymes.

Pharmacokinetics of MTX

- The actual mechanisms of action of low-to-moderate-dose MTX are still not fully understood, but it is thought that the anti-inflammatory effects, mediated by adenosine release, are more important than the antiproliferative effects.

Demographic, clinical & genetic predictors of MTX response & MTX-associated AEs

- A number of demographic, clinical factors and genes have been investigated for the association with MTX response and AEs, but the results between studies are very inconsistent and no definite conclusion can be drawn from these results published to date.

Methodology & study design

- Discrepancies between studies may partly be explained by differences in outcome measures, inclusion of possible confounders, independent factors evaluated and underpowered sample sizes.

Model

- The explained variance of models developed to date is relatively small.

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