

# Therapeutic drug monitoring of methotrexate in disease

Professor Dr Robert de Jonge, Dr Gerrit Jansen, Ittai Muller MSc and Helen Gosselt MSc, Amsterdam University Medical Center, are pioneering analytical approaches which can be used to understand why patients respond differently to the anchor drug used to manage rheumatoid arthritis, called methotrexate. Their work has begun to unravel the key factors behind the mechanism of action of methotrexate, involving compounds called methotrexate polyglutamates, and an enzyme called folylpolyglutamate synthetase. Ultimately, their research may lead to the development of personalised therapy for patients with rheumatoid arthritis and other diseases treated with this drug, including inflammatory bowel disease.

Methotrexate (MTX) is a folic acid analogue that has been used as a therapeutic agent for over 60 years. It works by stopping cells using folic acid to make building blocks for DNA synthesis, and is often prescribed to patients with certain types of cancers or inflammatory conditions, such as rheumatoid arthritis and inflammatory bowel disease (IBD).

Historically, higher doses of the drug were used to treat cancers, such as paediatric acute lymphoblastic leukaemia, some solid tumours, and maintenance therapy of childhood leukaemia often includes a low-dose oral MTX. Following on from this, methotrexate was then used at low doses to treat chronic autoimmune disorders such as rheumatoid arthritis and Crohn's disease.

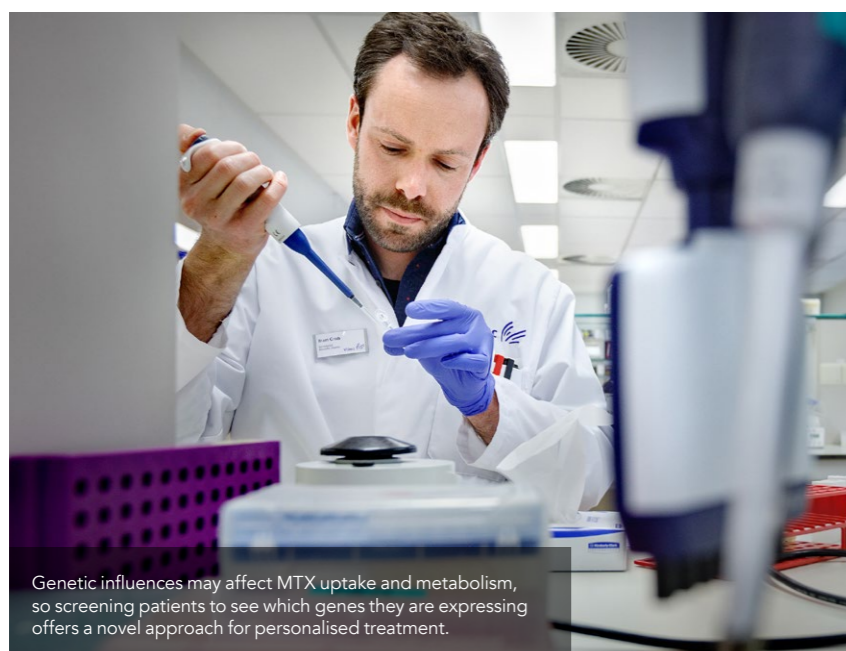
Due to its low cost, safety and effectiveness, in addition to its established clinical history, MTX is currently the first-line treatment for rheumatoid

arthritis. However, approximately 40% of patients with rheumatoid arthritis do not respond to MTX and do not show clinical improvement. Proposed reasons for non-response have included the variation between patients in MTX uptake and/or metabolism, non-compliance with the drug regimen or prescription of insufficient MTX dose. This lack of response may occur early in the treatment process or may occur after an initial response to MTX, after 3-6 months of therapy. These patients must then be prescribed more expensive second-line therapies.

Although a lack of response to MTX may be due to under-dosing, caution is required when deciding the dose of MTX as uncontrolled increases in dosage risk overdosing patients and may result in toxic side effects. Prediction models to select the right drug at the start of treatment and therapeutic drug monitoring for MTX during the initial stages of treatment may be a useful tool to identify patients who will or will not respond to the drug, and thus inform the correct dose for each individual patient.

There may be also genetic influences on MTX uptake and metabolism, so screening patients to see which genes they are expressing offers a novel approach for personalised treatment. Unfortunately, genetic associations with response to MTX treatment were not consistent, meaning that this is not currently a viable option for screening patients. Another limitation has been the lack of good quality analytical techniques that work on the cell types involved in rheumatoid arthritis.

The reasons for the differences in patient response to MTX are not fully understood; deepening our



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understanding of this is the focus of research of Professor Dr Robert de Jonge, Dr Gerrit Jansen, Ittai Muller MSc, Helen Gosselt MSc, and colleagues at Amsterdam University Medical Center.

## THE WAY THAT MTX WORKS

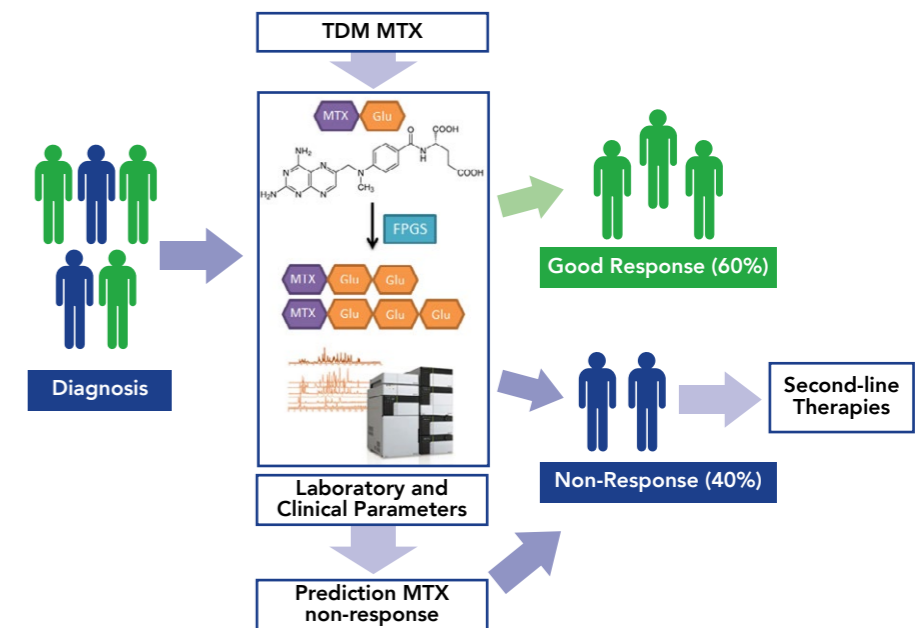
The mechanism of action of MTX overlaps with its clinical effectiveness in rheumatoid arthritis and cancer. Following uptake of MTX via a transport protein called the reduced folate carrier, an important step in the mechanism of action of MTX is the conversion of MTX to active metabolites called MTX-polyglutamates (MTX-PGs), by an enzyme called folylpolyglutamate synthetase (FPGS). These MTX-PGs cannot be exported by cells; and are therefore retained in the immune cells which are responsible for driving the inflammatory processes seen in rheumatoid arthritis. In addition, MTX-PGs enhance the potency of the drug by strongly inhibiting enzymes inside the cell. As these enzymes are normally involved in DNA synthesis, their inhibition results in reduced cell proliferation and activation. Fewer activated inflammatory cells means a reduction in clinical symptoms and pain in disease.

## THERAPEUTIC DRUG MONITORING

Therapeutic drug monitoring of MTX aims to measure the level of MTX-PGs in the blood cells of patients with rheumatoid arthritis and to correlate these levels with response to therapy. Professor Dr de Jonge anticipated that higher accumulation of MTX-PGs would promote a better response to drug therapy, and a lower level of MTX-PGs may be seen in the non-responding patients. Leading on from this, it could be hypothesised that the enzyme activity of FPGS could be important, as low FPGS activity would result in a decrease in conversion of MTX to MTX-PGs and hence a poorer response to therapy.

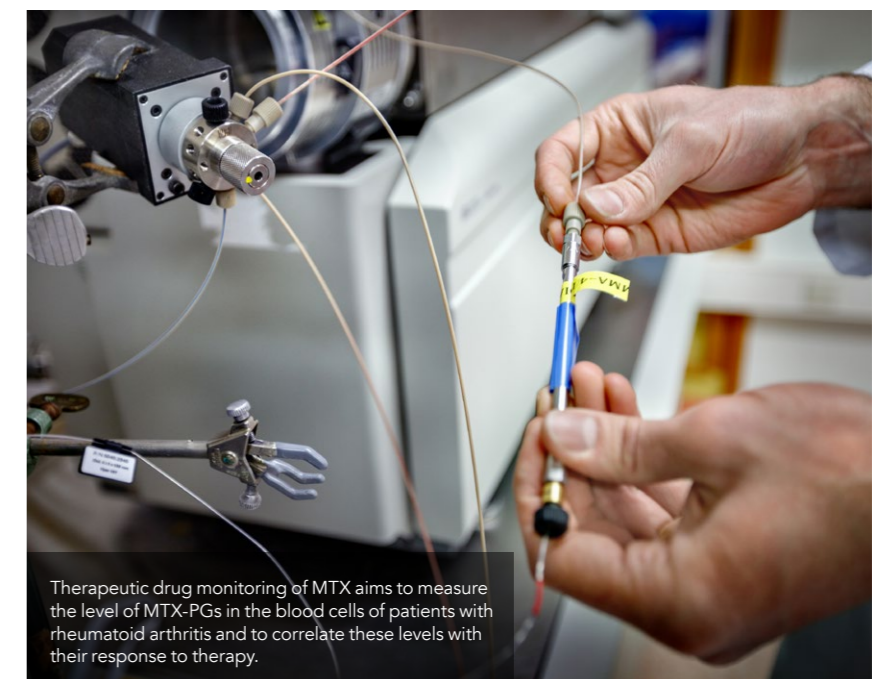
In order to achieve this, therapeutic drug monitoring of MTX has been carried out by analysing MTX-PG levels in red blood cells of patients with rheumatoid arthritis whilst they are undergoing MTX treatment. The analytical technique used

## Therapeutic Drug Monitoring (TDM) of Methotrexate (MTX) in disease



Methotrexate (MTX) is the anchor drug in the treatment of rheumatoid arthritis and other diseases. With respect to rheumatoid arthritis, clinical practice shows that 40% of the patients do not respond properly to this drug, thus challenging the identification of these patients so that they may switch to second-line therapies as early as possible. Therapeutic drug monitoring of MTX can be positioned as an analytical tool to measure methotrexate and its active metabolites, called methotrexate polyglutamates (MTX-PGs), in red blood cells and white blood cells of patients. The analytical tool employed is mass spectrometry which can measure MTX-PG levels with high sensitivity. Beyond this, the analytic tool can be used to measure the catalytic activity of the enzyme folylpolyglutamate synthetase (FPGS), responsible for the formation of MTX-PGs. Analyses of MTX-PGs and FPGS activity, along with other laboratory and clinical parameters, can help to distinguish those patients who do not respond to MTX.

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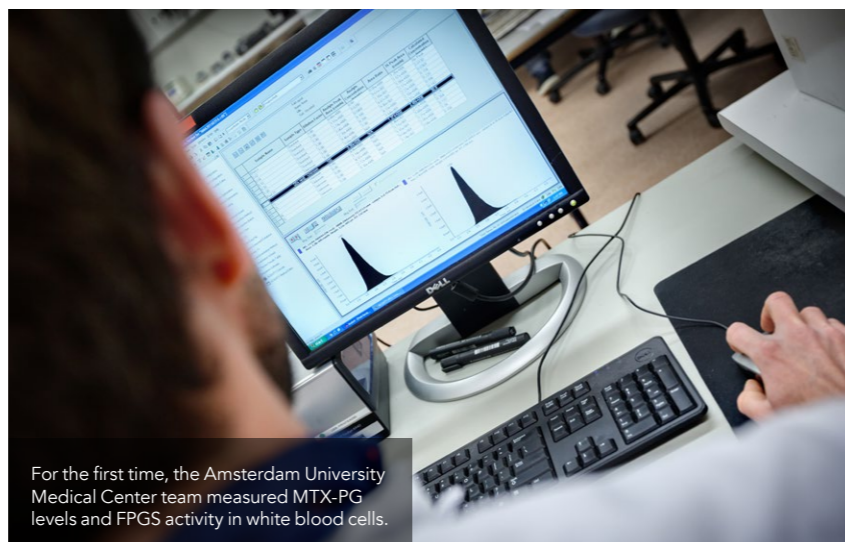


Therapeutic drug monitoring of MTX aims to measure the level of MTX-PGs in the blood cells of patients with rheumatoid arthritis and to correlate these levels with their response to therapy.





Higher MTX-PGs are associated with lower disease severity over nine months of MTX treatment.



For the first time, the Amsterdam University Medical Center team measured MTX-PG levels and FPGS activity in white blood cells.

is mass spectrometry, which is a way to identify molecules in a sample by using their mass to produce spectral patterns which are unique to particular molecules. The results showed that higher MTX-PGs were indeed associated with lower disease severity over nine months of MTX treatment, but that MTX-PGs were not associated with adverse effects. These findings provide support for the use of MTX-PG concentration in the therapeutic drug monitoring of MTX.

Previous studies showed that there was a huge variation in the level of MTX-PGs in red blood cells of patients who were all receiving the exact same dose of MTX to treat their rheumatoid arthritis. This highlights the need for personalised therapy regimens, designed to suit individual patients, rather than using a 'one size fits all' approach.

#### TARGETING THE RIGHT BLOOD CELLS

Measuring MTX-PG levels in red blood cells acts as a surrogate marker for MTX-PG levels in white blood cells, the cells which are involved in immune and inflammatory responses in rheumatoid arthritis. Thus, rather than measuring MTX-PGs in red blood cells, it would be more relevant to measure MTX-PG levels and FPGS activity directly in white blood cells, especially because loss of FPGS activity is associated with reduced MTX activity. Very recent work by the

group at Amsterdam University Medical Center has demonstrated the feasibility of analysing MTX-PG levels and FPGS activity in white blood cells using mass spectrometry, something that had not been done up until this point. Prior to this, assessment of FPGS activity and MTX-PG was carried out using assays based on radioactive substances, which were labour intensive and require relatively large numbers of cells. The advantages of using mass spectrometry are that no radioactive substances are required, and it has high sensitivity, even with low numbers of cells.

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#### FUTURE PLANS

Early effective treatment of rheumatoid arthritis is important to minimise irreversible damage to joints and long-term disability, therefore understanding more about the mechanisms behind response to MTX can help guide rheumatologists to personalise treatment based on whether or not the patient is identified as a responder or a non-responder to MTX.

Recently published work by Professor Dr de Jonge and colleagues includes an MTX-prediction model that is based on patient features such as age, gender, folate status and gene expression. The model also incorporates findings from

previous studies which showed that a higher MTX-PG level is associated with decreased disease severity, and that increasing the dose of MTX results in accumulation of MTX-PGs in red blood cells. This, alongside the novel mass spectrometry methods they have already established, paves the way for more research into the therapeutic drug response of MTX.

Professor Dr de Jonge suggests that future therapeutic drug monitoring studies may identify a target cut-off level for MTX accumulation in red blood cells and/or white blood cells. This would facilitate an optimal response to drug treatment as optimal MTX dose could be confirmed

earlier in the treatment process, leading to more efficient and safer use of MTX. In addition, therapeutic drug monitoring may help to avoid prescribing unnecessarily high doses of MTX to patients, as some may need lower doses than others to see improvements in clinical symptoms. Overall, therapeutic drug monitoring of MTX will produce data which will contribute to the implementation of prediction models for optimising and personalising MTX therapy in rheumatoid arthritis and other diseases and will help rheumatologists to prescribe the optimal MTX dose for maximum effectiveness and minimal side effects.

# Behind the Research



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## Research Objectives

Robert de Jonge's research aims to set up and implement decision algorithms for the right drug selection and therapeutic drug monitoring (TDM) modalities, combining state-of-the-art analytical, molecular and computational techniques to aid clinicians in optimising drug dosing for personalised therapy.

## Detail

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#### Bio

Robert de Jonge received his PhD in 1999 at the Erasmus University Rotterdam in 1999, followed by training and registry in Clinical Chemistry. He is now professor and head of the Department of Clinical Chemistry at the Amsterdam University Medical Center, directing research programmes on personalised drug treatment.

#### Funding

Dutch Arthritis Association, Dutch Society for Clinical Chemistry and Laboratory Medicine, Maag Lever Darm Stichting

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## Personal Response

**Do you think these techniques could be expanded for use in therapeutic monitoring of drugs other than methotrexate?**

Our approach is that in inflammatory diseases, first, one should select the right drug for the right patient at the start of treatment (at diagnosis) by using smart decision algorithms (prediction models for therapeutic drug response). Once the most optimal drug for the individual patient is selected, therapeutic drug monitoring of methotrexate or biologics will aid in reaching the desired anti-inflammatory effect.

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