

The Hematologic Toxicity of Methotrexate in Patients with Autoimmune Disorders

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Abstract

The incidence of auto-immune diseases is increasing now-a-days. Despite of the development of diagnosis and management of diseases, they remained chronic diseases. The patient's lifespan expansion requires long-term treatment with harmful agents, such as Methotrexate or other immuno-suppressive drugs. The Methotrexate toxicities are based on the duration and cumulative dosing of drug, and the combination with other drugs. Myelosuppression and consequent pancytopenia is the most frequent hematologic toxicity, which occur mostly later during low dose methotrexate administration.

We demonstrate three cases of low dose Methotrexate toxicity in older patients with rheumatoid arthritis and psoriasis.

All patients were treated with low dose Methotrexate along more than one year continuously.

Two old patients with RA and another with psoriasis developed pancytopenia causing severe neutropenia, cutaneous bleeding, and bruising and septic condition. They required intravenous antibiotic therapy, corticosteroids and limited transfusion dependence as a result of low dose Methotrexate.

We have assessed the possible causes of Methotrexate toxicities and found that all patients used non-steroid anti-inflammatory drugs because of pain and proton-pump inhibitor to avoid development of peptic ulcer. Two patients recovered, another died in septic condition.

We would like to draw attention of hematologists, dermatologists and rheumatologists to the harmful effect of low dose methotrexate in this patient population and emphasize the role of rigorous and consequent hematologic testing to avoid these severe late complications.

Keywords: Methotrexate; Psoriasis; Rheumatoid arthritis; Immunotherapy; Hematological toxicity

Introduction

The incidence of autoimmune diseases increased to the end of twenty centuries. Despite of the development of diagnosis and therapy in medicine, autoimmune diseases remained incurable. Only long-term treatment with harmful agents is available for these patients, such as methotrexate or other immuno-suppressive drugs.

Methotrexate, a synthetic antifolate was developed in the 1950's after the discovery that dietary folic acid deficiency resulted in decreased blast cell count in leukemic patients.

This drug has been extensively investigated and used successfully to treat solid tumours as well as hematological malignancies. It has been used, in much lower doses, in autoimmune diseases including rheumatoid arthritis, psoriasis, lupus, sarcoidosis, and eczema [1].

By inhibiting several enzymes of the folic acid pathway MTX blocks purine and pyrimidine biosynthesis, leading to impaired DNA replication and cell proliferation. Tissues with high cellular turnover are thus the most sensitive to the cytotoxic impact of Methotrexate, which is responsible for its effectiveness as a chemotherapeutic agent, but also for many of its side effects such as mucositis, hair loss and cytopenias.

Myelosuppression and pancytopenia including thrombocytopenia, megaloblastic anaemia, and leukopenia are the most frequent hematological toxicity, which occur later during low dose Methotrexate treatment. The hematologic toxicity is a serious and potentially life-threatening, still often underestimated complication of low dose MTX therapy. The pancytopenia often causes neutropenic sepsis. Low dose Methotrexate therapy can become dangerous, in particular with the elderly, who are at a greater risk for significant myelosuppression.

The prevalence of hematological toxicity is estimated to be 2% to 4% of all treated cases [2]. It may be much higher in the presence of predisposing factors: folic acid deficiency, hypoalbuminaemia, renal impairment and interaction with other drugs.

MTX induced pancytopenia can either occur in the early stage of treatment, possibly as a result of an idiosyncratic reaction [2]. The methotrexate late toxicities are based on the duration and cumulative dosing of drug, and probably the combination with other drugs.

In this paper, we present three cases with pancytopenia and neutropenic sepsis. The differential diagnosis, the treatment and possible drug interactions are also discussed.

Case Reports

Patient's characteristics

The main characteristics of the patients are listed in **Table 1**. All patients were treated with low dose MTX in another Hospital and were referred to our department because of pancytopenia. All patients had febrile neutropenia and septic condition.

The patients and relatives were asked particularly for anamnestic data. All patients were treated along more than one year with low dose MTX because of autoimmune disease. Peripheral blood smear of patients were examined and bone marrow aspiration were made to exclude any underlying hematological malignancy. No bone marrow infiltration or increased blast ratio were observed.

The diagnosis of MTX caused pancytopenia was based in all cases on the exclusion of previous conditions.

Table 1 Main characteristics of patients.

Variables	Patient 1	Patient 2	Patient 3
Age (years)	59	83	78
Diagnosis	RA	RA	psoriasis
Co-morbidity	HT, Asthma	HT, IHD	HT, DM, erosive gastritis
Possible drug-interactions	Theophyllin, Naproxen	Aspirin, pantoprasol	Pantoprasol, Gliclazide
Physical examination	oral mucositis, skin bruises	hypotension, confusion, urinary bleeding	oral mucositis
Antibiotics	Meropenem	Imipenem + Cilastatin and Vancomycin	Imipenem + Cilastatin
Hemoculture	neg	Enterococcus fecalis	neg
Estimated cost of hematologic therapy (in Eur)	4000	3500	4400
Abbreviations: RA: Rheumatoid Arthritis; HT: Hypertension; IHD: Ischemic Heart Disease; DM: Diabetes Mellitus.			

Case 1

Fifty nine-year-old female with a medical history of hypertension, asthma and rheumatoid arthritis had been

taking Methotrexate for 2 years in a weekly dose of 15 mg. She had been taking theophylline for her asthma in a daily dose of 300 mg and daily 550 mg Naproxen for her painful arthritis. She had 38°C fever two times the week before admission, and physical examination revealed oral mycositis with bruises all over her skin. Her vital signs were stable and her laboratory results showed pancytopenia (WBC: 2.09 g/l, neutrophils: 1.28 g/l) hemoglobin 63 g/l, haematocrit 20%, platelets 12 g/l), low serum protein level (51 g/l), high CRP (184.7 mg/l), with normal renal functions. Peripheral blood smear and bone marrow aspiration analysis ruled out haematological malignancies which underlied the suspicion that MTX induced myelosuppression was responsible for our findings.

We started empirical antibiotic and anti-mycotic therapy together with Ca-folate and parenteral corticosteroid. She also received red blood cell and platelet infusions.

After 12 days of therapy her symptoms improved significantly and her hematological parameters developed to almost normal (WBC: 4.28 g/l, neutrophils: 2.79 g/l, hemoglobin: 91 g/l, haematocrit: 27.3%, platelets 121 g/l).

Case 2

Eighty three-year-old female with a medical history of hypertension, ischaemic heart disease and rheumatoid arthritis had been taking Methotrexate for 16 months in a weekly dose of 16 mg subcutaneous injection (0.3 ml s.c./week). Patient had been taking aspirin for ischaemic heart disease in a daily dose of 100 mg and pantoprazole for peptic ulcer prophylaxis in a daily dose of 40 mg. She had 39.5°C fever before admission, and became confused in her home. Physical examination revealed tachycardia, hypotonia and tachypnoea and urinary bleeding. Her vital signs were instable and she was admitted in septic condition to our department. Her laboratory results showed pancytopenia (WBC: 1.02 g/l, neutrophils: 0.4 g/l) hemoglobin 83 g/l, haematocrit 27%, platelets 5 g/l), low serum albumin level (32 g/l), high procalcitonin level (13 µg/l), with decreased renal functions (Creatinine 189 µmol/L, GFR: 18 ml/min). Peripheral blood smear and bone marrow aspiration analysis excluded haematological malignancies. MTX induced myelosuppression was diagnosed. Empirical combined antibiotics and infusion were introduced with Ca-folate and parenteral corticosteroid. She also received platelet transfusions. Hemoculture was positive, *Enterococcus fecalis* developed. Despite of the accurate therapy, patient died after 2 days because of multiple organ failure.

Case 3

Seventy eight-year-old female with hypertension, diabetes, erosive gastritis and psoriasis had received Methotrexate 15 mg/week for 19 months when she was admitted to our department with febrile neutropenia. She had been taking pantoprazole for erosive gastritis in a daily dose of 40 mg and gliclazide for diabetes mellitus in a daily dose of 60 mg. On admission she had oral mucositis, her laboratory results showed severe myelo-suppression (WBC: 0.12 g/l, neutrophil: 0.02 g/l, hemoglobin 81g/l, haematocrit 22.4%, platelets 23

g/l), low total protein (45 g/l) and albumin (23 g/l) levels, elevated renal function (creatinine 114 $\mu\text{mol/l}$) and high CRP (296.9 mg/l). She was immediately started on a combination of parenteral antibiotics and local antimycotics, received corticosteroids, Ca-folate, red blood cell and platelet transfusions. On the 8th day of her treatment her cell counts started to normalize (WBC: 10.34 g/l, hemoglobin 102 g/l, platelets 44 g/l).

Discussion

Methotrexate is the commonest disease-modifying anti-inflammatory drug used in monotherapy or in combination with other drugs and biological agents the treatment of many autoimmune disorders. MTX, a highly selective competitive inhibitor of the enzyme dihydrofolate reductase. It consequently reduces the production of thymidylate and purine biosynthesis. DNA synthesis eventually halts and cells can no longer replicate [3].

The folate antagonist MTX also works on the adenosine pathway with important anti-inflammatory effects. Inhibition of transformylase by MTX leads to accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide and ultimately leads to increased levels of adenosine. Adenosine is a potent inhibitor of inflammation and induces vasodilation. This effect of MTX does not seem to be affected by folate supplementation [4]. The intracellular polyglutamination of MTX prolongs its intracellular presence, which contributes to their toxicity [5,6]. Adverse side effects can be quickly progressive and fatal. The main side effects include myelosuppression, hepatotoxicity, pneumonitis and renal toxicity.

Hematologic toxicity

The prevalence of hematologic toxicity, including leukopenia, thrombocytopenia, megaloblastic anaemia and pancytopenia, is estimated to be 2% to 4% [2]. The frequency of pancytopenia may increase if other drugs, such as non-steroidal anti-inflammatory drugs [NSAID], proton pump inhibitor [PPI] and antidiabetics are co-administered. The prevalence of pancytopenia may be increased in case of folic acid deficiency, hypoalbuminemia, concomitant infections, advanced age, dehydration and renal impairment [7].

Pathogenesis of MTX inducing pancytopenia is still unknown. Pancytopenia may be acute or chronic and thought to be an allergy-like reaction [8]. MTX and folates compete cellular uptake, cellular storage as polyglutamates and binding to enzymes, because of their structural similarity. Depleted intracellular folate levels have been documented in peripheral blood lymphocytes of RA patients treated with MTX [9]. Delayed drug clearance had been observed in the elderly. This is caused by prolonged enterohepatic circulation, which is responsive to higher risk of pancytopenia. Leukopenia occurs from one to three weeks and marrow recovery is generally observed within approximately 3 weeks [2,10-13].

Discontinuation of MTX represents the basis of therapy but the use of G-CSF and methylprednisolone are also beneficial [14].

Hepatotoxicity

Hepatotoxicity is a common complication of long term treatment with MTX [15,16], especially in the case of obesity, alcoholism, diabetes, non-alcoholic steatohepatitis and hepatitis B or C infection [17,18]. Elevated aminotransferases level is the most common laboratory sign of hepatotoxicity. It was observed in MTX-treated rheumatoid arthritis and psoriatic arthritis patients with a frequency varying from 7.5% to 26% [16]. The hepatotoxic side effect of the MTX is unclear. Folic acid supplementation is associated with a reduced incidence of aminotransferases elevation in inflammatory disease treated by MTX [19].

Pulmonary toxicity

Pneumonitis is one of the most serious but infrequent side effects of chronically used MTX. Its prevalence seems about 0.9% to 1% [20]. It has been thought, that a hypersensitivity reaction to MTX mediated by activated T-cells plays a role in the mechanism of pneumonitis [21]. In fact, MTX leads to a cytokines release by type 2 alveolar cells causing an alveolitis by recruitment of inflammatory cells [22].

MTX can also stimulate lung fibroblasts and epithelial cells to induce recruitment of eosinophil's [23]. It has been also demonstrated that neutrophils are implicated in the pathogenesis of lung fibrosis [24]. Cough, dyspnoea may occur from few days to more than a year after the beginning of MTX therapy and also several weeks after MTX discontinuation [25].

Renal toxicity

Renal toxicity occurs mostly in higher doses of MTX, especially intravenous administration of this drug. MTX and its metabolites are relatively insoluble in acid urine [26]. An increase in the urine pH results on a greater solubility of the drug and its metabolites. For that reason, it is recommended to monitor renal function time to time in order to control renal side effect especially in case of higher dose administration of MTX. However the serum creatinine may be a misleading measure of renal function in older patients because of an overall reduction in lean muscle mass. Urine alkalinisation and leucovorin rescue are the cornerstones of the management of the earlier signs of renal dysfunction [15,27].

Factors that contribute to the toxicity

Advanced age is a significant predictor for MTX toxicity [28]. The pharmacokinetic profile in elderly patients, change drug distribution. The decreased lean body mass and end-organ blood flow, and decreased hepatic drug metabolism, or decreased renal drug excretion lead to toxicity. MTX side effect can be increased in renal impairment or reduced renal blood flow, as with NSAIDs use [29]. Therefore MTX is contraindicated in any patient with eGFR <30 mL/min [28].

According to pharmacokinetic studies, about the 50% of MTX is bounded to serum albumin in the circulation (42% to 57%), whereas their metabolite (7-hydroxymethotrexate) is extensively (91% to 93%) bound. Significant interindividual variations in the activity of binding proteins lead to the efficacy and potential toxicity of MTX. The free portion of MTX determines the influx of MTX into cells and its rate of clearance by the kidneys. Hypoalbuminaemia results in increased levels of free MTX because MTX binding to serum albumin is proportional to the amount of albumin, resulting in increased risk of myelotoxicity. Hypoalbuminaemia in RA may be due to increased albumin turnover, presumably caused by high consumption of albumin at sites of inflammation and poor nutritional status. Occult chronic liver disease and advanced age may also be reflected in low serum albumin [29]. Poor nutritional status has been associated with increased risk of MTX toxicity.

Prevention and management of MTX toxicity

There are some general aspects of MTX administration and post-treatment management. To avoid MTX side effects, it is advised that routine blood count be performed every four to eight weeks [1] and it is mandatory to determine renal function time-to time. When renal function impaired, MTX dose adjustment is necessary. When creatinine clearance [CrCl] is between 30 and 60 ml/min, dose of MTX is reduced by 50% and when CrCl is under 30 ml/min, dose of MTX should be reduced by 75% [30]. Folic acid treatment [1 to 3 mg/day] after 2-3 days of MTX administration decreases the frequency of toxicities, such as mucositis, hematologic abnormalities and liver enzyme elevations, without seeming to interfere with clinical efficacy [31].

The most complicated prevention practice is the avoiding drug interactions. Most elderly patient take many drugs which having the potential to displace MTX from serum proteins and/or to reduce MTX clearance. The most known are interaction with trimethoprim and sulfamethoxazole [TMP-SMX] and NSAIDs [30,32,33].

Alteration of the elimination of MTX was also reported with antibiotics such as aminoglycosides, some penicillin's and macrolides [34], mezocilline [35], piperacillin [36], amphotericine B [37] and ciprofloxacin [38]. Proton pump inhibitors such as omeprazole [39,40], had same interactions.

Monitoring plasma MTX level is an essential part of high dose MTX therapy, but it is not necessary in low dose MTX treatment.

Our three cases demonstrated that the low dose MTX can cause easily life threatening complications. Monitoring for hematologic toxicity should be done every 4 to 8 weeks by primary care physicians but our patients had no blood sampling for 4 months. All patients had septic condition due to granulocytopenia and despite of adequate treatment one patient died in multiple organ failure in our department. Age is also a determinant of whether patients will survive pancytopenia and its associated complications, such as sepsis,

as the age of our dead patient was 83 years whereas the age of those who recovered from severe sepsis was lower.

We would like to draw attention to toxic effect of low dose Methotrexate of hematologists dermatologists and rheumatologists and primary care physicians.

Conclusion

Despite possible side effects of weekly administered low dose MTX used in autoimmune diseases, MTX is very well tolerated and its efficacy is excellent. When monitored correctly, the side effects can be avoided. It is very important that primary care physicians, the hematologists aware of these complications and recommendations, because the majority of these serious complications can be detected on time and even prevented. Patients on MTX therapy should be regularly monitored with renal and liver function and hematology tests to identify myelosuppression and avoid the sequela of pancytopenia.

More attention should be paid to patients' nutritional status especially the serum albumin level before commencing MTX. Folic acid supplementation should be considered in all patients taking MTX.

In our experience, MTX-induced pancytopenia is more common than expected and is probably under-reported. We recommend vigilance for this late and potentially fatal complication of MTX therapy.

Conflict of Interest

None of the authors have any conflict of interest to disclose.

Authorship

PR collected data, interpreted data and wrote the manuscript, VSK wrote the manuscript, ÁK collected data, ÉK performed physical examination and made table, BK wrote manuscript, ME designed case report and wrote the manuscript.

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