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Systematic Review and Meta-analysis

Cytopenias among patients with rheumatic diseases using methotrexate: a meta-analysis of randomized controlled clinical trials

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Abstract

Objective. To conduct a systematic literature review and meta-analysis to estimate the incidence of anaemia, leucopoenia, neutropenia and thrombocytopenia associated with MTX plus folic acid among patients with rheumatic diseases.
Methods. We searched MEDLINE, PubMed and EMBASE through August 2016 for all randomized controlled clinical trials with a MTX monotherapy arm. We excluded randomized controlled clinical trials for cancer and included only double-blind studies that reported on haematologic adverse events. Studies were excluded if patients did not receive folic acid or leucovorin supplementation. Full text articles were assessed by two independent reviewers. Incidence estimates were calculated using random-effects models.
Results. Of 1601 studies identified, 30 (1.87%) were included, representing 3858 patients; all had RA. Seventeen trials reported on anaemia ($n = 2032$), 17 reported on leucopoenia ($n = 2220$), 16 reported on neutropenia ($n = 2202$) and 12 reported on thrombocytopenia ($n = 1507$). The incidence for any anaemia was 2.55% (95% CI 0.60–5.47%), any leucopoenia 1.17% (95% CI 0.16–2.80%), any neutropenia 1.77% (95% CI 0.33–4.00%), and any thrombocytopenia 0.19% (95% CI 0.00–0.86%). Four cases of severe anaemia were reported, as defined by authors, along with three cases of severe neutropenia. No cases of severe leucopoenia, severe thrombocytopenia or pancytopenia were reported.
Conclusion. Cytopenias are an uncommon side effect of low-dose MTX with folic acid supplementation among RA patients. Further research is needed to reach a more precise estimate.

Key words: Methotrexate, rheumatoid arthritis, anaemia, leucopoenia, neutropenia, thrombocytopenia, meta-analysis

CLINICAL SCIENCE

Rheumatology key messages

- Incidence of anaemia was ~2.55%, of leucopenia was ~1.17%, of neutropenia was ~1.77%, of thrombocytopenia was ~0.19%.
- No cases of pancytopenia were reported across 30 trials representing 3858 individuals.
- Folic acid supplementation and modern clinical guidelines may account for the infrequency of cytopenias.

Introduction

Now a cornerstone in the treatment of RA, MTX was first used in oncology to treat haematologic cancers. MTX and its metabolites inhibit dihydrofolate reductase, which is required for the production of nucleic acids. Even at low dosages (7.5–25 mg weekly) used for rheumatic diseases, MTX can slow or halt the maturation of haematopoietic cells and reduce blood cell counts across all cell lineages [1, 2]. MTX recommendations from rheumatology societies suggest complete blood counts every 2–3 months as a necessary part of clinical monitoring [3–5] .

In the 1990s, folic and folinic acid (FA) supplementation became part of standard practice as a means of combating cytopenias and other MTX-associated adverse events. However, since the widespread use of FA, the incidence of cytopenias among patients taking low-dose MTX has not been re-examined. Such data may improve the rationale for evidence-based monitoring guidelines for MTX.

We conducted a systematic literature review and meta-analysis to estimate the incidence of anaemia, leucopoenia, neutropenia and thrombocytopenia associated with MTX plus FA supplementation among patients with

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rheumatic diseases enrolled in randomized controlled clinical trials.

Methods

We searched PubMed, MEDLINE and EMBASE databases through August 2016 without specifying a start date and using the search terms 'methotrexate' and either 'pancytopenia', 'anaemia', 'leukopenia', 'thrombocytopenia' or 'drug-related side effects and adverse reactions'. We excluded studies with 'neoplasms' as a Medical Subject Heading (MeSH). Full PubMed search criteria are available in the online supplement (Supplementary Tables S1 and S2, available at *Rheumatology* online). We reviewed all resulting records and removed those without abstracts available in English. From these abstracts we included only double-blind randomized controlled clinical trials in adults. We excluded abstracts from oncology studies and included only those studies that studied inflammatory diseases. As folic and FA supplementation became part of standard practice in the late 1990s, studies published prior to the year 2000 were excluded if they did not specify that all subjects were taking FA supplementation. Those published after the year 2000 were considered to include FA supplementation for all patients unless otherwise specified.

We manually reviewed the reference sections of all included papers for additional studies that met our selection criteria. We then reviewed full-text articles and included only those with a MTX monotherapy arm. Studies that allowed concomitant use of other synthetic DMARDs (e.g. hydroxychloroquine) with MTX were excluded. Concomitant use of glucocorticoids was allowed. Only articles that included reports of the occurrence and type of haematologic adverse events were included.

Study characteristics, adverse event data, and risk of bias assessment were carried out by two independent authors (K.V. and D.H.S.) using a predefined data abstraction form, which is available in the online supplement. Risk of bias was assessed according to the Cochrane Collaboration guidelines for random sequence generation and allocation concealment (selection bias), blinding of participants and researchers (performance bias), blinding of outcome assessment (detection bias), study retention (attrition bias), and selective or incomplete reporting (reporting bias) [6]. Discrepancies were resolved through discussion.

The primary outcomes were the occurrence of any of the following: anaemia, leucopoenia, neutropenia and thrombocytopenia. Each was defined according to the Outcome Measures in Rheumatoid Arthritis Clinical Trial (OMERACT) standards [7] or per report of the trial authors. Leucopoenia and neutropenia were considered independent of one another.

Random-effects models were examined for the estimate of the incidence for each outcome. We included articles in the analyses of each cytopenia if they reported the occurrence or absence of the four main cytopenias; each study was included in one to four analyses. The incidence was calculated as the number of positive cases divided by the total sample size of relevant studies. The arcsine square root transformation was used to stabilize the weights of estimates. Estimates are represented using a diamond. The pattern of the diamond represents the estimated effect size and the width of the diamond reflects the precision of the estimate. The incidence was then estimated for each cytopenia among a subgroup of studies that included MTX-naïve patients. The meta-analysis was analysed and graphed using R-3.4.3 (https://cran.r-project.org) with the 'meta' package.

Study heterogeneity was tested using the Cochran's Q and I^2. I^2 is the percentage of observed total variation across studies due to real heterogeneity, a value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. The Cochran's Q is the weighted sum of squares on a standardized scale. The 0.10 is used as a cut-off for significance of presence of heterogeneity. Possible publication bias was investigated graphically using funnel plot and Egger's weighted regression statistic with a P-value <0.05 indicating significant publication bias [8].

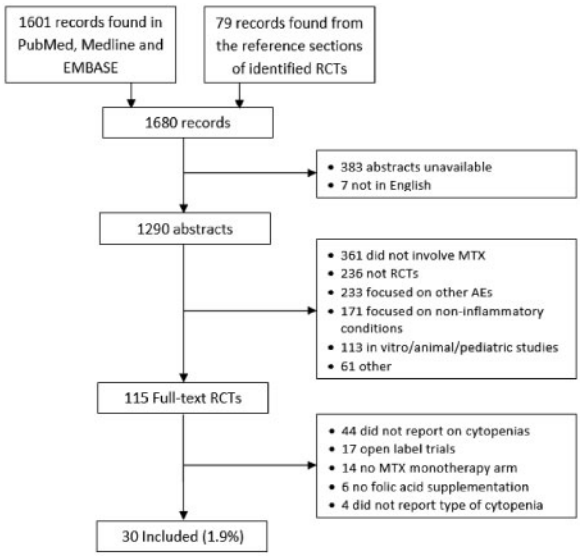
Results

PubMed, MEDLINE and EMBASE searches yielded 1601 records (Fig. 1) . After excluding records without abstracts available in English, 1211 abstracts were reviewed; 28 of those met inclusion criteria and were reviewed as full text articles. After review of the reference sections of these 28 articles, another 79 studies were identified. Forty-four full text articles were excluded because they did not report on haematologic adverse events. Another four were excluded for reporting haematologic abnormalities in aggregate without specifying the type of cytopenia. Thirty studies were included in our analyses.

The included studies represented 3858 patients randomized to MTX monotherapy. In 26 studies ($n = 3278$), subjects took MTX plus a placebo or a biologic disease modifying anti-rheumatic drug (bDMARD). Two studies ($n = 224$) randomized subjects to MTX or leflunomide monotherapy. Three studies ($n = 356$) assigned subjects to MTX plus FA or placebo. Though we did not search or filter by disease, all included trials studied RA; no other conditions were represented.

Seventeen trials reported rates of anaemia ($n = 2032$), 12 reported on thrombocytopenia ($n = 1507$), 17 reported on leucopoenia ($n = 2220$), 16 reported on neutropenia ($n = 2202$), and five reported on lymphopenia ($n = 324$). Among the 22 studies ($n = 2926$) that reported MTX doses, the mean dose of MTX was 15.4 (S.D. 4.5) mg/week, with a maximum dose of 30 mg/week (Table 1). Seventeen studies ($n = 2666$) reported on the percentage of subjects using corticosteroids, with 41.9% of subjects using corticosteroids. Study duration ranged from 12–62 weeks with a mean of 31 (S.D. 16) weeks. Fifteen studies reported on the route of MTX administration, of which ten used oral MTX only and five used oral or parenteral MTX.

FIG. 1 Study selection process per PRISMA guidelines



We analysed the five cytopenia separately, and each analysis included only the studies that reported that type of cytopenia as having occurred or not. A total of 64 cases of anaemia were reported across 17 studies ($n = 2032$; see Fig. 2A). The incidence of any anaemia by a random effects model was 2.55% (95% CI 0.60–5.47%). Of these cases, 18 were mild (27.7%), seven were moderate (10.8%), three were severe (4.6%), one was life threatening (1.5%), and 35 were of unknown severity (53.8%).

Forty-one cases of leucopoenia occurred across 17 studies ($n = 2220$; see Fig. 2B); one case was reported as mild (2.4%), and severity was not reported for the remaining 40 cases. The incidence of any leucopoenia was 1.17% (95% CI 0.16–2.80%). Among 16 studies ($n = 2202$), there were 65 cases of neutropenia (see Fig. 2C). The incidence of neutropenia was 1.77% (95% CI 0.33–4.00%). Twenty-nine cases were reported as mild neutropenia (44.6%), nine were moderate (13.8%), three were severe (4.6%), none were life threatening (0%), and 24 (36.9%) were not characterized by severity.

Thrombocytopenia was seen in eight cases across 12 studies ($n = 1507$; see Fig. 2D), with two cases being reported as mild (25%) and the other six cases being of unspecified severity. The incidence of any thrombocytopenia was 0.19% (95% CI 0.00–0.86%). No studies reported any cases of pancytopenia.

Six studies included only MTX-naïve subjects [9, 10, 14, 29, 31, 35]. Among this subgroup ($n = 1034$), one study reported no occurrences of anaemia [14] ($n = 160$) and one reported 19 cases of neutropenia [10]($n = 217$). Three studies [9, 29, 35] ($n = 475$) reported a total of 21 cases of leucopoenia. The incidence of leucopoenia among this MTX-naïve subgroup was 4.16% (95% CI 1.01–8.97%). One case of thrombocytopenia was reported between two studies [31, 35] ($n = 456$) in the MTX-naïve subgroup; the incidence of thrombocytopenia among this subgroup was 0.13% (95% CI 0.00–1.07%). Forest plots for the leucopoenia and thrombocytopenia subanalyses are shown in Fig. 3A and B.

Significant heterogeneity existed across studies for all cytopenias. The I^2 was 89% for anaemia ($P < 0.01$), 81% for leucopoenia ($P < 0.01$), 86% for neutropenia ($P < 0.01$) and 42% for thrombocytopenia ($P = 0.06$). Cochran's Q was 140.69 ($P < 0.01$) for anaemia, 85.84 ($P < 0.01$) for leucopoenia, 109.59 ($P < 0.01$) for neutropenia and 18.91 ($P = 0.06$) for thrombocytopenia.

As double-blind studies, all studies had a low risk of performance and detection bias. Twenty-three studies did not describe their method of random sequence generation, and 19 studies did not report their strategy for allocation concealment. Eight studies experienced loss to follow-up and did not report on adverse events for all subjects. Another eight studies only reported on serious adverse events or events that led to study discontinuation. Risk of bias assessment is detailed in Fig. 4.

The publication bias of the primary outcomes was assessed using visual examination of funnel plots and Egger's weighted regression statistic ($P = 0.084$ for anaemia, $P = 0.073$ for leucopoenia, $P = 0.654$ for neutropenia and $P < 0.001$ for thrombocytopenia [Fig. 5A–C]), which indicated a potential publication bias for thrombocytopenia. Trim-and-fill results suggested that 13 more studies would be needed to achieve a symmetry funnel plot, as shown in Fig. 5D.

Discussion

MTX has long been known to increase the risk of cytopenias, but the incidence of haematologic abnormalities among patients taking low-dose MTX in the era of FA supplementation has remained poorly defined. We conducted the first systematic review and meta-analysis to our knowledge to estimate the incidence of cytopenias among RA patients taking low-dose MTX with FA. We identified 30 double-blind randomized clinical trials that reported on haematologic adverse events in such a population. The incidence was 2.55% (95% CI 0.60–5.47%) for anaemia, 1.17% (95% CI 0.16–2.80%) for leucopoenia, 1.77% (95% CI 0.33–4.00%) for neutropenia, and 0.19% (95% CI 0.00–0.86%) for thrombocytopenia.

While low red blood cell folate levels are associated with elevated rates of cytopenias in patients taking low-dose MTX [39, 40], FA supplementation has not been directly associated with fewer cases of cytopenias. Three meta-analyses have looked for a reduction in haematologic abnormalities during FA supplementation but failed to find any due to small sample sizes and inconsistent reporting [1, 41, 42]. Estimates of the incidence of haematologic abnormalities associated with low-dose MTX published prior to the widespread use of folic acid supplementation ranged from as low as 3% [43, 44] to as high as 10% [45, 46] combined across all cell lines. Our results are consistent with these lower estimates.

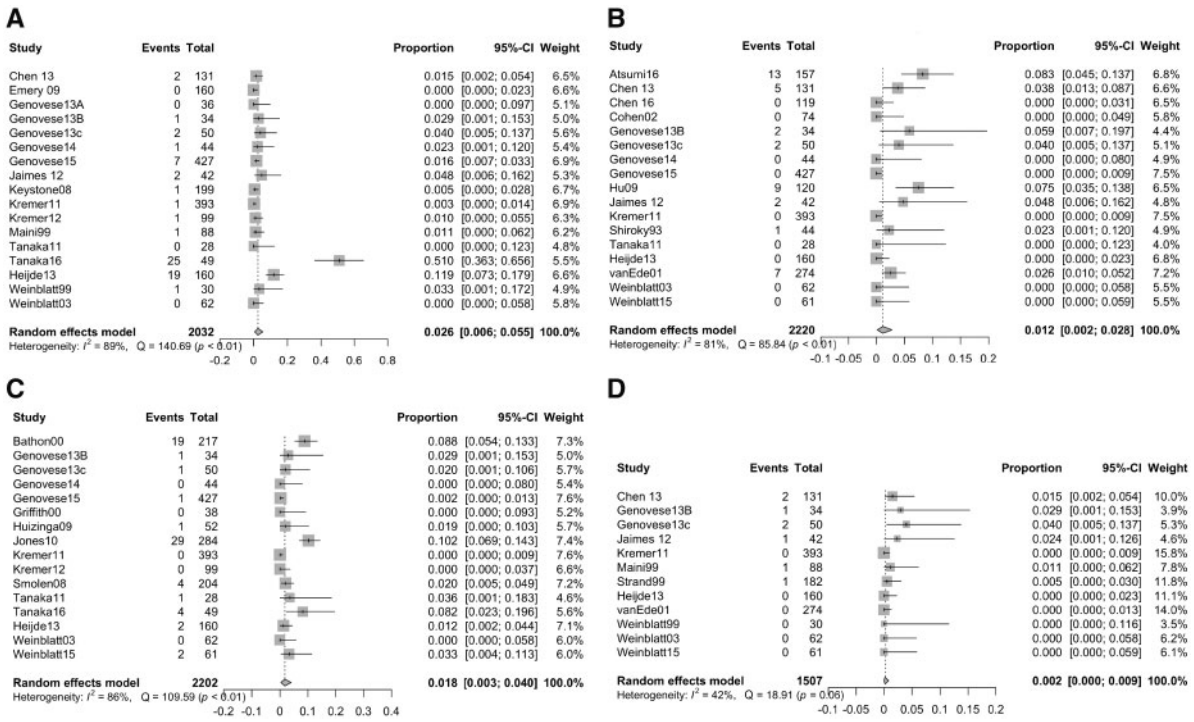
Current professional resources list the incidence of thrombocytopenia to be between 3–10%; however,

Table 1 Characteristics of included trials

Author	Year	Setting	Region	Comparator drug	MTX mono, *n*	Mean Dose of MTX, mg/week	Route of MTX	Folate or Leucovorin	% Taking steroids	Length of trial, weeks	Frequency of CBCs
Atsumi [9]	2016	Multi	Asia	Certolizumab	157	11.6	Oral	—	18.50%	52	—
Bathon [10]	2000	Multi	N. America	Etanercept	217	19.0	Oral	Folic, 1mg/day	18.90%	52	2 weeks, 1 month, 6, 8, 10, 12 months
Chen [11]	2013	Multi	Asia	Anbainuo	131	16.7	Oral	—	27.50%	24	—
Chen [12]	2016	Multi	Asia	Anbainuo	119	—	—	—	—	12	Weeks 2, 6, 12
Cohen [13]	2002	Multi	N. America, Asia	Anakinra	74	16.3	—	—	66.20%	24	Weeks 0, 1, 2, 4, 8, 12, 16, 20, 24
Emery [14]	2009	Multi	Global	Golimumab	160	19.1	—	—	68.10%	24	—
Genovese [15]	2013	Multi	N. America	Tabalumab	36	16.8	—	—	—	24	—
Genovese [16]	2013	Multi	Europe	Tabalumab	34	12	—	—	41%	24	—
Genovese [17]	2013	Multi	Global	Sekukinumab	50	—	—		—	16	Week 2, 4, 8, 16
Genovese [18]	2014	Multi	N. America, Europe	Olokizumab	44	—	—	—	—	12	—
Genovese [19]	2015	Multi	Global	Sarilumab	427	15.6	Oral	Folic	63.30%	62	Weeks 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 36, 42, 50
Griffith [20]	2000	Single	Europe	Placebo Folate	38	13.35	—	Folic, 5mg/day	23.68%	52	Every 3 months
Hu [21]	2009	Multi	Asia	Etanercept	120	12.5	Oral	—	28.33%	24	Weeks 2, 4, 8, 12, 16, 20, 24
Huizinga [22]	2013	Multi	Global	Sarilumab	52	16.9	—	Folic	—	12	Every 2 weeks
Jaimes-Hernández [23]	2012	Single	N. America	Leflunomide	42	10	Oral	—	—	52	Weeks 4, 8, 16, 24, 32, 40, 48, 52
Jones [24]	2010	Multi	Global	Tocilizumab	284	—	Oral	—	47%	24	—
Keystone [25]	2008	Multi	Global	Certolizumab	199	13.4	—	—	—	52	Weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52
Kremer [26]	2011	Multi	Global	Tocilizumab	393	15.0	Oral, PAR	—	11.50%	52	Weeks 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52
Kremer [27]	2012	Multi	Global	Tofacitinib	99	17.0	Oral, PAR	Folic	44.90%	24	Weeks 2, 4, 6, 8, 12, 16, 20, 24
Maini [28]	2000	Multi	N. America, Europe	Infliximab	88	15	Oral, PAR	Folic	64%	30	—
Shiroky [29]	1993	Multi	N. America	PBO Leucovorin	44	—	Oral	Folinic, 2.5-5mg/day	—	52	Every 3 weeks
Smolen [30]	2008	Multi	Global	Tocilizumab	204	14.8	—	Folic	54%	24	Weeks 2, 4, 6, 8, 12, 14, 16, 20, 24, 28, 32
Strand [31]	1999	Multi	N. America	Leflunomide	182	—	Oral	Folic	52.70%	52	—
Tanaka [32]	2011	Multi	Asia	Tofacitinib	28	8.1	—	Folic	71.40%	12	Weeks 1, 2, 4, 8, 12
Tanaka [33]	2016	Multi	Asia	Baricitinib	49	—	—	—	—	12	—
van der Heijde [34]	2013	Multi	Global	Tofacitinib	160	—	—	—	—	12	—
van Ede [35]	2001	Multi	Europe	PBO Folate	133	18.0	Oral	Folic, 1 mg/day	—	48	Every 3 weeks
					141	16.4	Oral	Folinic, 2.5mg/week			
Weinblatt [36]	1999	Multi	N. America	Etanercept	30	18	Oral, PAR	Folic or Folinic	70%	24	Days 1, 8, 15 weeks 4, 8, 12, 16, 20, 24
Weinblatt [37]	2003	Multi	N. America	Adalimumab	62	16.5	Oral, SC	Folic	—	24	—
Weinblatt [38]	2015	Multi	Global	Clazakizumab	61	17.5	—	Folic	—	24	Weeks 1, 2, 4, 8, 12, 16, 20

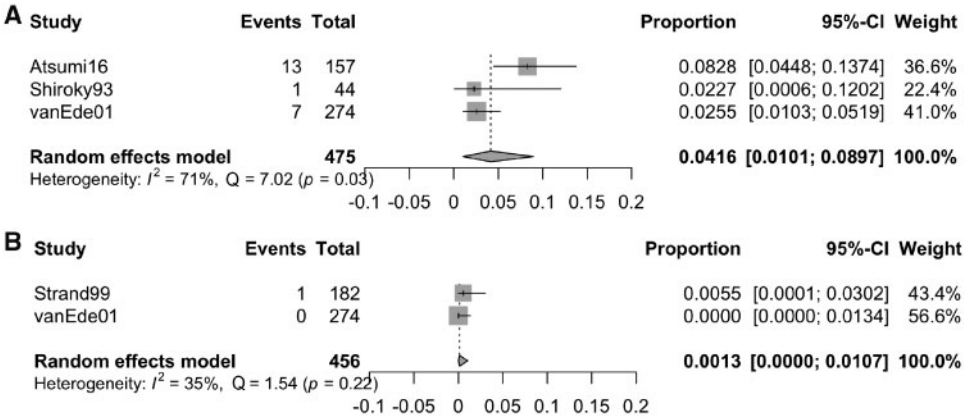
CBC, complete blood count; Multi, multicentre; —, not specified; N. America, North America; PAR, parenteral; PBO, placebo; SC, subcutaneous.

FIG. 2 Incidences of (**A**) anaemia, (**B**) leucopoenia, (**C**) neutropenia and (**D**) thrombocytopenia



Forest plot showing incidence of each cytopenia across all studies that reported on its presence or absence at any point during the study. The grey squares represent the weight of each study, and the black bars show the 95% CIs. The grey diamond shows the incidence.

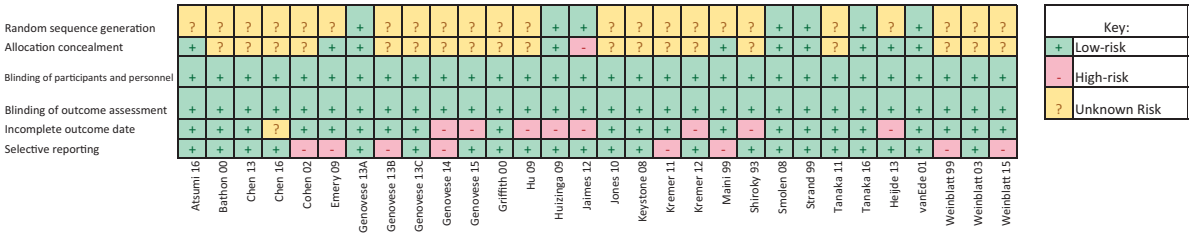
FIG. 3 Incidences of (**A**) leucopoenia and (**B**) thrombocytopenia in studies that included MTX-naïve subjects



Forest plot showing incidence of (**A**) leucopoenia and (**B**) thrombocytopenia among studies that included MTX-naïve subjects. The grey squares represent the weight of each study, and the black bars show the 95% CIs. The grey diamond shows the incidence.

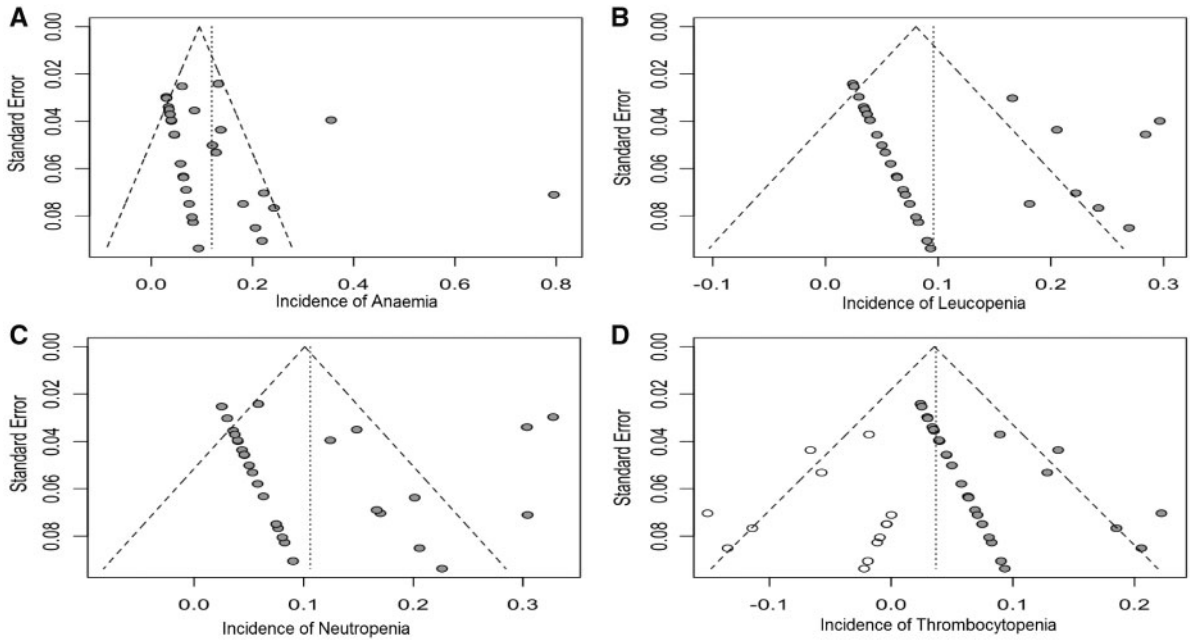
these estimates are drawn from studies published prior to the widespread use of FA supplementation [39, 47, 48]. Other prior studies have estimated that thrombocytopenia occurs in about 4.1–4.7% [49, 50] of patients. In contrast, we found thrombocytopenia to be the least common cytopenia with an incidence of <1%. Thrombocytopenia appears to be a rare side effect of low-dose MTX in the era of FA supplementation.

Fig. 4 Cochrane risk of bias assessment



Author assessment of the risk of bias for each included study.

Fig. 5 Funnel plots for (**A**) anaemia, (**B**) leucopoenia, (**C**) neutropenia and (**D**) thrombocytopenia



Risk of publication bias analysis for each type of cytopenia.

Prior literature has reported that pancytopenia occurs in 1.4–2% [47, 51] of patients taking low-dose MTX for RA, while resources for physicians list the incidence of pancytopenia in this population to be between 1–3%. However, among 3858 patients represented in our analysis, no cases of pancytopenia were reported. It remains possible that cases of multiple line cytopenia or pancytopenia were reported as multiple events that each affected one cell line. Pancytopenia appears to be very rare in patients without prior risk factors who receive close clinical monitoring.

Several factors limited our analysis. First, few studies stated the precise definitions or the severity of the cytopenias that they reported, and definitions varied across studies. The high I^2 values for each analysis point to significant heterogeneity across all studies. Second, doses of FA and corticosteroids varied and may have influenced the rates of cytopenias. Corticosteroids raise white blood cell counts and may decrease the rates of leucopoenia and neutropenia. All studies required FA supplementation, and most studies noted 10 mg prednisone daily as the maximum allowable dose of corticosteroids.

Third, the trials in our analysis included a relatively healthy subset of RA patients. For example, Atsumi and colleagues, Shiroky and colleagues, and Tanaka and colleagues set age limits of 65, 70 and 75 years, respectively [9, 29, 33], although about two-thirds of the included trials did not comment on an upper age limit. Kremer and colleagues and van der Heijde and colleagues required an estimated glomerular filtration rate of at least 50 ml/min and 40 ml/min, respectively, and Weinblatt and colleagues required a creatine level of <177 μmol/l [27, 34, 36]. However, most studies did not specify requirements for kidney function. Thus, the elderly and those with renal insufficiency were likely to be excluded from these studies and may be more likely to develop cytopenias.

Finally, most of the included studies compared MTX with a biologic, so to be eligible for these studies, patients must have tolerated MTX prior to the trial period without developing cytopenias. Our results reflect the rates of cytopenias in patients established on MTX and FA, but these rates may be higher in those initiating MTX.

In a subanalysis of MTX-naïve subjects, we found that the rate of leucopoenia was 4.16% (95% CI 1.01–8.97%) among MTX-naïve subjects, compared with 1.17% (95% CI 0.16–2.80%) among all subjects. Leucopoenia appears to be more common in those who recently started MTX, though more data would be needed to confirm this finding. The incidence of thrombocytopenia was 0.13% (95% CI 0.00–1.07%) among the MTX-naïve subgroup and 0.19% (95% CI 0.00–0.86%) among all subjects. Thrombocytopenia does not seem to be more common among MTX-naïve patients. More data would be needed to evaluate the frequency of cytopenias in patients newly started on MTX.

Our study had several important strengths. First, by including only double-blind randomized trials, we minimized the risks of selection and performance bias. Second, subjects enrolled in a clinical trial receive laboratory monitoring at regular intervals, which allows for the consistent detection of cytopenias. Third, the use of FA supplementation by all subjects in all included studies agrees with current practice. Finally, we meta-analyzed 30 studies total, which gave our analyses a relatively robust sample size.

In conclusion, cytopenias are uncommon among patients with RA taking low-dose MTX with FA supplementation. In patients established on MTX, anaemia, leucopoenia and neutropenia occur much less frequently than previously estimated. Thrombocytopenia is a rare side effect of low-dose MTX, occurring far less frequently than previously thought. Similarly, pancytopenia is far less common than previously estimated, which could be due to the universal use of FA supplementation and the establishment of clear contraindications to MTX due to the risk of pancytopenia (e.g. renal failure, active infections, concomitant use of sulfamethoxazole-trimethoprim). Given the low incidence of cytopenias among patients with no other risk factors taking low-dose MTX, current monitoring guidelines might be reconsidered.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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