Review Article

Off-Label Uses of Methotrexate

Indrani Sarma[1], Dibyajyoti Saikia[1]

1. Department of Pharmacology, AIIMS Guwahati, India

Methotrexate (MTX), originally developed as a chemotherapeutic agent, has evolved into a versatile immunomodulatory drug with extensive off-label applications across multiple medical specialties. This study synthesizes evidence of the use of Methotrexate in dermatology, gastroenterology, obstetrics and gynecology, rheumatology, ophthalmology, hematology, and emerging applications in infectious diseases and cardiovascular medicine. The evidence base reveals that while MTX demonstrates efficacy in numerous off-label indications. Dermatological applications are supported by low-quality evidence across 31 conditions, while obstetric uses for ectopic pregnancy show 91% efficacy in appropriate clinical scenarios. Gastroenterological applications in Crohn's disease are well-established, though evidence for ulcerative colitis remains limited. Emerging applications in COVID-19 treatment and cardiovascular risk reduction represent promising areas requiring further investigation. Safety profiles are generally acceptable at low doses, though monitoring for hepatotoxicity, hematologic abnormalities, and pulmonary complications remains essential.

Corresponding author: Indrani Devi Sarma, i.sarma17@gmail.com

1. Introduction

Methotrexate (MTX) represents an example of successful drug repurposing, having transitioned from its original indication as an antineoplastic agent to become a cornerstone therapy in numerous inflammatory and autoimmune conditions. While MTX is approved for specific indications including rheumatoid arthritis, psoriasis, and certain malignancies, its immunomodulatory and anti-inflammatory properties have led to widespread off-label use across diverse medical specialties [1]. Off-label prescribing, defined as the use of an authorized drug for indications not described in the Summary of Product Characteristics, is legally permissible when physicians can justify treatment based on available scientific evidence [2].

 doi.org/10.32388/QWZNDA

The expanding scope of MTX applications reflects both its unique pharmacological profile and the clinical need for effective, cost-accessible therapies for inflammatory conditions. However, the evidence supporting many off-label uses varies considerably in quality and quantity, raising important questions about optimal prescribing practices, dosing strategies, and safety monitoring.

Methotrexate exerts its therapeutic effects through multiple interconnected mechanisms that extend beyond its classical role as a dihydrofolate reductase inhibitor. At the low doses typically employed in inflammatory conditions (5-25 mg weekly), MTX demonstrates immunomodulatory activity through adenosine signalling modulation, alteration of cytokine networks, generation of reactive oxygen species, and suppression of the HMGB1 alarmin [3]. These anti-inflammatory mechanisms are distinct from the antiproliferative effects observed at higher chemotherapeutic doses.

The drug's antiatherogenic properties include improved endothelial function, slower atherosclerosis progression, and decreased risk of major cardiovascular adverse events, positioning MTX as a potential therapeutic agent for cardiovascular risk reduction in inflammatory diseases [4]. Additionally, MTX exhibits antiangiogenic effects through inhibition of macrophage invasion during early angiogenesis and suppression of endothelial cell proliferation, which has implications for ophthalmological and oncological applications [5].

The pharmacokinetics of MTX vary significantly based on route of administration, with parenteral (intramuscular or subcutaneous) formulations demonstrating superior bioavailability compared to oral administration, particularly at doses exceeding 15 mg weekly [6]. This pharmacokinetic variability has important implications for clinical efficacy across different indications.

2. Off-Label Indications

2.1. Dermatological Applications

Dermatology represents one of the most extensively studied areas for off-label MTX use, though paradoxically, the evidence quality remains suboptimal. A comprehensive systematic review employing the GRADE methodology evaluated MTX efficacy across 31 dermatological diseases, analyzing 143 studies involving 3,688 patients [2]. The review concluded that evidence for effectiveness, efficacy, and safety was uniform across all examined conditions.

Atopic Dermatitis

Atopic dermatitis represents a particularly well-studied off-label indication, with five randomized controlled trials demonstrating MTX efficacy in improving disease signs and symptoms despite considerable heterogeneity in dosing regimens [7]. Starting doses ranged from 7.5-15 mg weekly in adults, with maintenance doses of 14.5-25 mg weekly. Pediatric dosing followed weight-based or body surface area-based protocols, with starting doses of 10-15 mg/m^2/week and maintenance doses of 0.2-0.7 mg/kg/week [7]. The lack of consensus on optimal dosing regimens represents a significant barrier to standardized clinical practice.

2.2. Obstetric and Gynecological Applications

Ectopic Pregnancy

Methotrexate has become a standard medical management option for ectopic pregnancy, despite its off-label status for this indication. The drug is administered systemically or locally, sometimes in combination with potassium chloride, to terminate ectopic pregnancies and avoid aggressive surgical interventions [8]. Efficacy varies by anatomical location and clinical parameters.

For cervical ectopic pregnancies, systemic MTX demonstrates 91% efficacy, with superior outcomes observed in pregnancies less than 9 weeks gestation and with human chorionic gonadotropin (hCG) levels below 10,000 mIU/mL [8]. For cesarean scar pregnancies, systemic MTX is indicated primarily for non-viable cases, particularly when hCG levels are below 5,000 mIU/mL [8].

Multiple clinical guidelines have been developed to standardize MTX use in ectopic pregnancy. The French National College of Gynecologists and Obstetricians (CNGOF) has published comprehensive guidelines addressing evidence-based evaluation and expert recommendations for off-label MTX use in gynecology and obstetrics [9]. These guidelines provide detailed protocols for patient selection, dosing regimens, and monitoring strategies.

A pharmacovigilance study based on the China Hospital Pharmacovigilance System evaluated safety in 672 patients receiving MTX for ectopic pregnancy [10]. The total incidence of adverse drug reactions was 54.0%, with anemia (37.6%), hepatic function abnormalities (11.3%), and hyperuricemia (6.1%) being most frequent [10]. Importantly, 86.3% of adverse events were CTCAE grade one (mild), 12.1% were grade two

(moderate), and only 1.6% were grade three (severe) [11]. Two-dose exposure significantly increased the risk of adverse events (OR 1.87), hepatic dysfunction (OR 2.75), and dyslipidemia (OR 5.15) [10].

Absolute contraindications for MTX in ectopic pregnancy include unknown pregnancy location, peptic ulcer disease, intrauterine pregnancy, MTX sensitivity, breastfeeding, suspected tubal rupture, and immunodeficiency [8].

Placenta Accreta Spectrum

The use of MTX in placenta accreta spectrum (PAS) disorders represents a more controversial application. A narrative review assessed MTX efficacy in treating PAS based on pregnancy trimester, noting limited consensus regarding safety or effectiveness [12]. The review investigated fertility-preserving alternatives, though specific quantitative efficacy data remain sparse. This application requires careful risk-benefit assessment and should be considered investigational pending higher-quality evidence.

2.3. Gastroenterological Applications

Crohn's Disease

Methotrexate has established efficacy in Crohn's disease (CD), supported by well-designed randomized controlled trials. The landmark North American Crohn's Study Group trial demonstrated that 39.4% of patients receiving MTX 25 mg intramuscularly weekly achieved clinical remission (CDAI <150) with prednisone discontinuation, compared to 19.1% in the placebo group at 16 weeks [11]. For maintenance therapy, MTX 15 mg intramuscularly weekly maintained steroid-free remission in 65% of patients versus 39% with placebo at 40 weeks [11].

A systematic review stratifying MTX outcomes by clinical indication found that high-quality evidence supports MTX use only in steroid-dependent CD, with efficacy comparable to thiopurines [13]. Evidence for other indications—including steroid-refractory disease, thiopurine failure, combination therapy with biologics, fistulizing disease, mucosal healing, and postoperative prevention—derives primarily from small, uncontrolled series [13]. Parenteral MTX at higher doses (25 mg weekly) during induction demonstrated superior performance across most indications [13].

Ulcerative Colitis

Evidence for MTX efficacy in ulcerative colitis (UC) remains limited and controversial. While MTX has proven effective in steroid-dependent CD, therapeutic options for steroid-dependent UC are currently restricted to azathioprine/6-mercaptopurine and infliximab [11]. A systematic review exploring MTX efficacy in UC patients found insufficient evidence to support routine use [11]. The limited efficacy in UC compared to CD may reflect differences in disease pathophysiology or inadequate investigation of optimal dosing and administration protocols.

Methotrexate is positioned as a second-line immunomodulating therapy in inflammatory bowel disease, typically reserved for patients intolerant or resistant to azathioprine or 6-mercaptopurine (affecting approximately 27-50% of refractory CD patients) [6]. The slow onset of clinical efficacy associated with purine analogues has prompted investigation of MTX as an alternative, though its role remains secondary to first-line agents and biologics in current treatment algorithms [14].

2.4. Rheumatological and Autoimmune Applications

Systemic Lupus Erythematosus

The role of MTX in systemic lupus erythematosus (SLE) remains incompletely defined, with conflicting evidence from clinical trials. Twenty uncontrolled case series and one retrospective cohort study support MTX use for active skin and joint disease in SLE [15]. However, three prospective randomized trials reported conflicting results: two demonstrated improvement in overall disease activity and decreased corticosteroid requirements, while the third showed no benefit for disease activity but did report corticosteroid-sparing effects [15]. These inconsistencies reflect the inherent challenges in conducting trials in moderately active SLE, including disease heterogeneity, variable outcome measures, and difficulties in patient stratification [15].

Viral Arthritis

Methotrexate has been investigated for chronic viral arthritis associated with alphaviruses, Parvovirus B19, hepatitis B/C virus, and human immunodeficiency virus [3]. These rheumatic syndromes often resemble rheumatoid arthritis clinically. MTX demonstrates effectiveness and good tolerability in these conditions, with efficacy mediated through adenosine signaling modulation, cytokine network alteration, reactive oxygen species generation, and HMGB1 alarmin suppression [3]. However, safety

concerns exist regarding potential attenuation of immune surveillance capacities in patients with chronic viral infections [3].

2.5. Ophthalmological Applications

Thyroid Eye Disease

A retrospective comparative case series of 36 patients evaluated MTX for active thyroid eye disease (TED) in patients unable to tolerate corticosteroids [16]. MTX significantly improved Clinical Activity Scores (7-CAS) with 94% of patients showing reduction at 12 months ($P < 0.0001$) [12]. Ocular motility disturbances improved in 67% of affected patients ($P < 0.001$) [12]. However, MTX showed no significant improvement in visual acuity, exophthalmos (mean 24 mm, SD 3 mm), or eyelid position (mean 6 mm, SD 1.5 mm) [12]. Notably, no side effects were registered, indicating good tolerability and confirming glucocorticoid-sparing effects [16].

Corneal Angiogenesis

Topical MTX application has been investigated for inhibition of corneal angiogenesis in experimental models. In a rabbit corneal pocket model with fibroblast growth factor-induced neovascularization, topical MTX (0.2 mg/day) reduced vascularized area from 12.0±6.9 mm^2 in controls to 2.2±1.86 mm^2 in treated eyes by day 9 [5]. Therapeutic levels were achieved in aqueous humor without detectable serum levels, and no local side effects such as epithelial defects were observed [5]. The antiangiogenic mechanism likely involves inhibition of macrophage invasion during early angiogenesis and endothelial cell proliferation [5]. These findings suggest potential applications for topical MTX in anterior segment inflammations, though clinical translation requires further investigation.

Ocular Surface Disease

A recent two-part study combining in vitro toxicity assessment and in vivo clinical evaluation investigated topical MTX for recalcitrant ocular surface disease, specifically inflammatory dry eye disease [17]. While the study aimed to determine safety and therapeutic potential, specific quantitative efficacy and safety data were not detailed in available abstracts, indicating this remains an area of active investigation.

2.6. Hematological Applications

Large Granular Lymphocyte Leukemia

Low-dose oral MTX has demonstrated efficacy in treating large granular lymphocyte (LGL) leukemia, a T-cell malignancy [18]. In a small series, three of five patients achieving complete clinical remission showed undetectable abnormal T-cell receptor gene rearrangement clones [18]. This application represents an important therapeutic option for a rare hematological malignancy with limited treatment alternatives.

Idiopathic Granulomatous Hepatitis

A case series of seven patients evaluated low-dose oral pulse MTX therapy for idiopathic granulomatous hepatitis in patients who had complications from, did not respond to, or refused alternative treatments [19]. Patients were counseled to promptly report respiratory symptoms (cough or shortness of breath), practice contraception, and avoid alcohol [19]. While specific quantitative outcomes were not detailed in available abstracts, this application demonstrates MTX utility in rare hepatic inflammatory conditions.

2.7. Emerging Applications

COVID-19 Treatment

An innovative bioinformatics approach combined with hamster model validation identified MTX as a potential repurposed drug for COVID-19 treatment [20]. The computational analysis predicted 327 therapeutic targets and 21,233 drug-target interactions for 1,592 FDA-approved drugs [3]. Experimental validation demonstrated that MTX potently inhibited SARS-CoV-2 replication with an EC50 of 0.4 μM and showed activity against all four variants of concern [20].

In the hamster model (n=12, divided into 4 groups of 3), MTX reduced virus replication and inflammation in lungs, improved body weight loss, and decreased total inflammation area by approximately 30% [20]. The drug demonstrated favorable selectivity with a cytotoxicity CC50 greater than 100 μM, yielding a selectivity index of approximately 250 [20]. These preclinical findings are promising, though clinical translation requires careful evaluation in patients with comorbidities and consideration of immunosuppressive effects during active infection.

Cardiovascular Risk Management

Methotrexate has emerged as a candidate for cardiovascular risk reduction in patients with inflammatory diseases. Approximately 20% of patients with atherosclerotic cardiovascular disease experience recurrent events despite maximal pharmacological treatment, highlighting residual cardiovascular risk [21]. MTX's unique combination of anti-inflammatory, blood pressure-lowering, and vasculoprotective effects positions it as a potential repurposed therapy for cardiovascular risk management [21].

Clinical trials have demonstrated that MTX is associated with improved endothelial function, slower atherosclerosis progression, decreased risk of major cardiovascular adverse events, and survival benefits [4]. These cardiovascular effects are mediated through antiproliferative, immunosuppressive, anti-inflammatory, and antiatherogenic mechanisms [4]. While pending results from large prospective studies investigating surrogate endpoints and clinical outcomes, MTX repurposing for cardiovascular risk management represents a potentially cost-effective strategy with immediate public health benefits [21].

Urticarial Vasculitis

A case report documented complete resolution of urticarial vasculitis with low-dose oral MTX in a patient unresponsive to all treatments except systemic corticosteroids [22]. The MTX therapy enabled corticosteroid withdrawal, demonstrating a steroid-sparing effect [22]. Low-dose MTX appears to carry an acceptable safety profile for this indication, though evidence is limited to case reports [22].

3. Dosing Regimens and Routes of Administration

Route-Dependent Efficacy

The route of MTX administration critically influences clinical efficacy, particularly in gastroenterological applications. Parenteral (intramuscular or subcutaneous) administration demonstrates superior efficacy compared to oral administration, especially at doses exceeding 15 mg weekly [6], [11]. This difference reflects the saturable intestinal absorption of oral MTX, which limits bioavailability at higher doses.

In Crohn's disease, intramuscular MTX 25 mg weekly achieved 39.4% remission versus 19.1% placebo, while oral MTX at lower doses failed to demonstrate significant efficacy [11]. For maintenance therapy, intramuscular MTX 15 mg weekly maintained remission in 65% versus 39% placebo [11]. These findings

underscore the importance of parenteral administration for optimal outcomes in inflammatory bowel disease.

Dosing

Dermatology: Adult starting doses range from 5-15 mg weekly, with maintenance doses of 7.5-25 mg weekly [7]. Pediatric dosing employs body surface area-based protocols: starting doses of 10-15 mg/m²/week, maintenance doses of 0.2-0.7 mg/kg/week [7]. Approximately half of guidelines recommend folic acid supplementation, and some suggest test dosing [7].

Obstetrics: Dosing for ectopic pregnancy varies by protocol (single-dose, two-dose, or multi-dose regimens) and is guided by hCG levels, gestational age, and anatomical location [9][8]. Two-dose exposure increases adverse event risk (OR 1.87) [10].

Gastroenterology: Induction therapy for Crohn's disease typically employs 25 mg intramuscularly weekly, with maintenance at 15 mg intramuscularly weekly [13], {m/26/}. Higher doses (25 mg weekly) during induction show superior performance across most indications [13].

Rheumatology: Low-dose protocols (typically 7.5-25 mg weekly) are standard for autoimmune conditions, with dosing individualized based on disease activity and tolerability [3][15].

Ophthalmology: Topical application for corneal angiogenesis employed 0.2 mg/day [5]. Systemic dosing for thyroid eye disease followed standard low-dose protocols [16].

Folic Acid Supplementation

Folic acid supplementation is recommended by approximately half of dermatology guidelines to mitigate MTX-related folate depletion and reduce adverse events [7]. This practice is widely adopted across specialties, though specific supplementation protocols vary. Standard recommendations typically include folic acid 1-5 mg daily or folinic acid (leucovorin) for rescue in cases of toxicity.

4.Safety

Common Adverse Events

The adverse event profile of low-dose MTX is well-characterized across multiple specialties:

Hematological: Anemia (37.6% in ectopic pregnancy cohort) [10], bone marrow suppression (dose-dependent)

Hepatic: Hepatic function abnormalities (11.3% in ectopic pregnancy cohort) [10], transaminase elevations, rare hepatic fibrosis with chronic use

Metabolic: Hyperuricemia (6.1% in ectopic pregnancy cohort) [10], dyslipidemia (increased risk with two-dose exposure, OR 5.15) [11]

Gastrointestinal: Nausea, mucositis, diarrhea (common but usually mild)

Pulmonary: Pneumonitis (rare but potentially serious), requiring prompt recognition and MTX discontinuation [19]

Dermatological: Rash, photosensitivity, alopecia

Absolute Contraindications

Contraindications vary by indication but generally include:

Universal contraindications: Pregnancy (teratogenic), breastfeeding, severe hepatic or renal impairment, bone marrow hypoplasia, immunodeficiency, active infection, hypersensitivity to MTX

Indication-specific contraindications for ectopic pregnancy: Unknown pregnancy location, peptic ulcer disease, intrauterine pregnancy, suspected tubal rupture [8]

Monitoring Protocols

Comprehensive monitoring is essential for safe MTX use. Complete blood count, comprehensive metabolic panel including liver and renal function, hepatitis B and C screening, chest radiograph (if pulmonary symptoms) should be done for baseline assessment.

Patients on long term use should be prescribed complete blood count and liver function tests every 2-4 weeks initially, then every 8-12 weeks once stable; renal function monitoring every 8-12 weeks

Prompt reporting of respiratory symptoms (cough, shortness of breath) [19], avoidance of alcohol [19], contraception requirements [19], recognition of infection symptoms should be part of communication and patient counselling.

5. Discussion

This review reveals a complex landscape of off-label MTX use characterized by widespread clinical adoption. Several key themes emerge from the synthesis of available evidence. Despite MTX being commonly prescribed for various dermatological conditions, a comprehensive systematic review found uniformly low-quality evidence across all indications [2]. This paradox reflects the historical evolution of MTX use based on clinical experience and case series rather than rigorous controlled trials. The situation is further complicated by the ethical and practical challenges of conducting placebo-controlled trials for conditions where MTX has become standard practice.

The critical importance of route of administration and dosing represents a consistent theme across specialties. The failure of oral MTX trials in Crohn's disease contrasted with the success of parenteral administration highlights the need for route-specific evidence evaluation [11]. Similarly, the wide variation in dosing regimens for atopic dermatitis (starting doses 5–15 mg weekly, maintenance 7.5–25 mg weekly) reflects the absence of dose-optimization studies [7].

One benefit is MTX's consistent steroid-sparing effect. This property is documented in Crohn's disease [11], systemic lupus erythematosus [15], thyroid eye disease [16], urticarial vasculitis [22], and various dermatological conditions [2]. Given the well-established adverse effects of chronic corticosteroid therapy, MTX's ability to reduce or eliminate steroid requirements represents a significant clinical benefit even in situations where direct disease-modifying effects are modest.

The high efficacy in ectopic pregnancy (91% for cervical ectopic) [8] likely reflects the drug's antiproliferative effects on rapidly dividing trophoblastic tissue. The differential efficacy in Crohn's disease versus ulcerative colitis [11] may indicate distinct immunopathogenic mechanisms. Understanding these specialty-specific patterns could inform patient selection and guide investigation of novel indications.

The investigation of MTX for COVID-19 treatment [20] and cardiovascular risk reduction [21], [4] exemplifies the ongoing potential for drug repurposing based on mechanistic insights. The bioinformatics approach that identified MTX as a SARS-CoV-2 inhibitor represents a modern methodology for systematic drug repurposing [20]. Similarly, the recognition of MTX's cardiovascular benefits emerged from observational studies in rheumatoid arthritis patients, leading to hypothesis-

driven investigation of cardiovascular applications [4]. These examples demonstrate how real-world evidence can identify novel therapeutic applications.

6. Conclusion

Methotrexate exemplifies successful drug repurposing, with extensive off-label applications across multiple medical specialties driven by its unique immunomodulatory and anti-inflammatory properties. High-quality evidence supports MTX use in ectopic pregnancy (91% efficacy for cervical ectopic pregnancies) and steroid-dependent Crohn's disease (39.4% remission rate, 65% maintenance of steroid-free remission), while dermatological applications, despite involving 31 conditions and 3,688 patients across 143 studies, are supported only by low-quality evidence.

The consistent steroid-sparing effects observed across specialties—including Crohn's disease, systemic lupus erythematosus, thyroid eye disease, and various dermatological conditions—represent a significant clinical benefit given the well-established adverse effects of chronic corticosteroid therapy. Route of administration critically influences efficacy, with parenteral formulations demonstrating superior outcomes compared to oral administration, particularly at higher doses. Dosing regimens vary substantially across and within specialties, reflecting the paucity of dose-optimization studies.

The safety profile of low-dose MTX is generally acceptable, with predominantly mild-to-moderate adverse events including anemia (37.6%), hepatic dysfunction (11.3%), and hyperuricemia (6.1%) in obstetric applications. Comprehensive monitoring protocols including baseline assessment and ongoing surveillance of hematological, hepatic, and renal function are essential for safe use. Absolute contraindications include pregnancy, breastfeeding, severe organ impairment, and immunodeficiency, with indication-specific contraindications for ectopic pregnancy.

Emerging applications in COVID-19 treatment and cardiovascular risk reduction represent promising areas for future investigation. Preclinical data demonstrate potent SARS-CoV-2 inhibition (EC50 0.4 μM) with approximately 30% reduction in lung inflammation in hamster models. Cardiovascular applications are supported by mechanistic rationale and observational data showing improved endothelial function, slower atherosclerosis progression, and reduced major adverse cardiovascular events, though large prospective trials are pending.

The evidence synthesis presented in this review provides a comprehensive foundation for evidence-based off-label MTX prescribing while highlighting the urgent need for methodologically rigorous

research to optimize clinical practice. As MTX continues to demonstrate utility across diverse indications, the medical community must balance pragmatic clinical use informed by decades of experience with the imperative to generate high-quality evidence supporting standardized, safe, and effective treatment protocols. The future of off-label MTX use lies in translating real-world experience into rigorously evaluated, guideline-supported clinical practice that maximizes therapeutic benefits while minimizing risks across diverse patient populations and clinical contexts.

References

1. ^*Sarma I, Barman B, Sarma P, Saikia D (2024). "Methotrexate Induced Lymphadenitis: A Case Report." Qeios. doi:10.32388/WYVZA5.2.*
2. a, b, c, d*van Huizen AM, Vermeulen FM, Bik CMJM, Borgonjen R, Karsch SAT, Kuin RA, Gerbens LAA, Spuls PI (2021). "On Which Evidence Can We Rely When Prescribing Off-Label Methotrexate in Dermatological Practice? – A Systematic Review with GRADE Approach." J Dermatolog Treat. **33**(4):1947–1966. doi:10.1080/09546634.2021.1961999.*
3. a, b, c, d, e, f*Bedoui Y, Guillot X, Sélambarom J, Guiraud P, Giry C, Jaffar-Bandjee MC, Ralandison S, Gasque P (2019). "Methotrexate an Old Drug with New Tricks." Int J Mol Sci. **20**(20):5023. doi:10.3390/ijms20205023.*
4. a, b, c, d, e*Bălănescu AR, Bojincă V, Bojincă M, Donisan T, Bălănescu S (2018). "Cardiovascular Effects of Methotrexate in Immune-Mediated Inflammatory Diseases (Review)." Exp Ther Med. doi:10.3892/etm.2018.6992.*
5. a, b, c, d, e*Joussen AM, Kruse FE, Völcker H-E, Kirchhof B (1999). "Topical Application of Methotrexate for Inhibition of Corneal Angiogenesis." Graefes Arch Clin Exp Ophthalmol. **237**(11):920–927. doi:10.1007/s004170050387.*
6. a, b, c*Sun JH, Das KM (2005). "Low-Dose Oral Methotrexate for Maintaining Crohn's Disease Remission." J Clin Gastroenterol. **39**(9):751–756. doi:10.1097/01.mcg.0000177249.46130.a3.*
7. a, b, c, d, e, f, g*Caron AGM, Bloem M, El Khattabi H, de Waal AC, van Huizen AM, Denswil NP, Gerbens LAA, Spuls PI (2023). "The Wide Variety of Methotrexate Dosing Regimens for the Treatment of Atopic Dermatitis: A Systematic Review." J Dermatolog Treat. **35**(1). doi:10.1080/09546634.2023.2292962.*
8. a, b, c, d, e, f, g*Parker VL, Srinivas M (2016). "Non-Tubal Ectopic Pregnancy." Arch Gynecol Obstet. **294**(1):19–27. doi:10.1007/s00404-016-4069-y.*

9. a, b Marret H, Fauconnier A, Dubernard G, Misme H, Lagarce L, Lesavre M, Fernandez H, Mimoun C, Tourette C, Curinier S, Rabishong B, Agostini A (2016). "Overview and Guidelines of Off-Label Use of Methotrexate in Ectopic Pregnancy: Report by CNGOF." Eur J Obstet Gynecol Reprod Biol. **205**:105–109. doi:10.1016/j.ejogrb.2016.07.489.
10. a, b, c, d, e, f, g Wang L, Cai C, Huang L, Shi D, Chen Q, Chen Y, Yu W (2023). "Safety Profile of Methotrexate Used Off-Label in Ectopic Pregnancy: An Active Monitoring Study Based on a Chinese Hospital Pharmacovigilance System." Curr Med Res Opin. **40**(1):35–42. doi:10.1080/03007995.2023.2285375.
11. a, b, c, d, e, f, g, h, i, j, k, l Herfarth HH, Osterman MT, Isaacs KL, Lewis JD, Sands BE (2010). "Efficacy of Methotrexate in Ulcerative Colitis." Inflamm Bowel Dis. **16**(8):1421–1430. doi:10.1002/ibd.21246.
12. a, b, c, d Nohra L, Nafeh G, Assi M, El Chbib D (2025). "The Use of Methotrexate in the Management of Placenta Accreta Spectrum: A Narrative Review." Ann Med Surg. **87**(9):5637–5643. doi:10.1097/ms9.0000000000003524.
13. a, b, c, d, e Cassinotti A, Batticciotto A, Parravicini M, Lombardo M, Radice P, Cortelezzi CC, Segato S, Zanzi F, Cappelli A, Segato S (2022). "Evidence-Based Efficacy of Methotrexate in Adult Crohn's Disease in Different Intestinal and Extraintestinal Indications." Ther Adv Gastroenterol. **15**. doi:10.1177/17562848221085889.
14. ^ Bernstein CN, Shanahan F (1993). "Immunosuppressive and Immunomodulatory Therapy for Inflammatory Bowel Disease." Can J Gastroenterol. **7**(2):115–120. doi:10.1155/1993/980602.
15. a, b, c, d, e Wong JM, Esdaile JM (2005). "Methotrexate in Systemic Lupus Erythematosus." Lupus. **14**(2):101–105. doi:10.1191/0961203305lu2043rr.
16. a, b, c, d Strianese D, Iuliano A, Ferrara M, Comune C, Baronissi I, Napolitano P, D'Alessandro A, Grassi P, Bonavolontà G, Bonavolontà P, Sinisi A, Tranfa F (2014). "Methotrexate for the Treatment of Thyroid Eye Disease." J Ophthalmol. **2014**:1–5. doi:10.1155/2014/128903.
17. ^ Seto NA, Wong CW, Lu X, Watsky MA, Yee RW (2025). "Tolerability and Efficacy of Topical Methotrexate in Ocular Surface Disease." Cureus. doi:10.7759/cureus.80958.
18. a, b Loughran TP Jr, Kidd PG, Starkebaum G (1994). "Treatment of Large Granular Lymphocyte Leukemia with Oral Low-Dose Methotrexate." Blood. **84**(7):2164–2170. doi:10.1182/blood.v84.7.2164.2164.
19. a, b, c, d, e, f Knox TA, Kaplan MM, Gelfand JA, Wolff SM (1995). "Methotrexate Treatment of Idiopathic Granulomatous Hepatitis." Ann Intern Med. **122**(8):592–595. doi:10.7326/0003-4819-122-8-199504150-00006.
20. a, b, c, d, e, f Chen YT, Chang YH, Pathak N, Tzou SC, Luo YC, Hsu YC, Li TN, Lee JY, Chen YC, Huang YW, Yang HJ, Hsu NY, Tsai HP, Chang TY, Hsu SC, Liu PC, Chin YF, Lin WC, Yang CM, Wu HL, Lee CY, Hsu HL, Liu YC, Chu JW, Wang LHC, Wang JY, Huang CH, Lin CH, Hsieh PS, Wu Lee YH, Hung YJ, Yang JM (2022). "Methotrexate

Inhibition of SARS-CoV-2 Entry, Infection and Inflammation Revealed by Bioinformatics Approach and a H amster Model." Front Immunol. ***13****. doi:10.3389/fimmu.2022.1080897.*

21. a, b, c, d *Mangoni AA, Tommasi S, Zinellu A, Sotgia S, Carru C, Piga M, Erre GL (2018). "Repurposing Existing Drugs for Cardiovascular Risk Management: A Focus on Methotrexate." Drugs Context.* ***7****:1–12. doi:10.7573/dic.212557.*
22. a, b, c, d *Stack PS (1994). "Methotrexate for Urticarial Vasculitis." Ann Allergy.* ***72****(1):36–38.*

Declarations

Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.