BMJ Open

# FOLIC ACID AND METHOTREXATE USE AND THEIR ASSOCIATION WITH COVID-19 DIAGNOSIS AND MORTALITY: AN ANALYSIS FROM THE UK BIOBANK



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2022-062945 |
| Article Type: | Original research |
| Date Submitted by the Author: | 25-Mar-2022 |
| Complete List of Authors: | Topless, Ruth; University of Otago<br>Green, Ralph; University of California Davis at Sacramento, Departments of Pathology and Medicine<br>Morgan, Sarah; The University of Alabama at Birmingham<br>Robinson, Philip ; University of Queensland Faculty of Medicine and Biomedical Sciences<br>Merriman, Tony; University of Otago, School of Medical Sciences<br>Gaffo, Angelo; The University of Alabama at Birmingham; Birmingham VA Medical Center |
| Keywords: | COVID-19, Epidemiology < INFECTIOUS DISEASES, Rheumatology < INTERNAL MEDICINE |
| | |

*I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.*

*The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.*

*Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.*

# FOLIC ACID AND METHOTREXATE USE AND THEIR ASSOCIATION WITH COVID-19 DIAGNOSIS AND MORTALITY: AN ANALYSIS FROM THE UK BIOBANK

Ruth K Topless BSc [1], Ralph Green MD PhD FRCPath[2] , Sarah L. Morgan MD RD[3], Philip C Robinson MBChB PhD [4], Tony R Merriman PhD[1,3] , Angelo L. Gaffo MD MsPH FACP [3,5]

[1]Department of Biochemistry, University of Otago, Dunedin, New Zealand.

[2]Departments of Pathology and Medicine, University of California Davis at Sacramento, Sacramento, California, USA.

[3]Division of Clinical Immunology and Rheumatology, The University of Alabama at Birmingham, Birmingham, Alabama, USA.

[4]School of Clinical Medicine, University of Queensland, Brisbane, Australia

[5]Birmingham VA Medical Center. Birmingham, Alabama, USA

**Word count in this version: 3481**

**Corresponding Authors and reprint requests:**

Angelo Gaffo

SHEL 306

1720 2nd Ave South

Birmingham, AL 35294.

USA

Phone: +1-205-9348909

Fax: +1-205-9966788

agaffo@uabmc.edu

Ralph Green

4400 V St.

Sacramento, CA 95817

Phone: +1-916-7348078

Fax: +1-916 – 7340299

rgreen@ucdavis.edu

**Abstract**

**Objective**: to determine if methotrexate or folic acid prescription were associated with differential risk for COVID-19 diagnosis or mortality.

**Design**: Case-control analysis.

**Setting**: the population-based UK Biobank (UKBB) cohort.

**Participants**: Data from 380,380 UKBB participants with general practice prescription data for 2019 to 2021. Updated medical information was retrieved on the 13th December 2021.

**Primary and secondary outcome measures**: The outcomes of COVID-19 diagnosis and COVID-19 related mortality were analyzed by multivariable logistic regression. Exposures evaluated were prescription of folic acid and/or methotrexate. Criteria for COVID-19 diagnosis were 1) a positive SARS-CoV-2 test or 2) ICD-10 code for confirmed COVID-19 (U07.1) or probable COVID-19 (U07.2) in hospital records, or death records. By these criteria 26,003 individuals were identified with COVID-19 of whom 820 were known to have died from COVID-19. Logistic regression statistical models were adjusted for age sex, ethnicity, Townsend deprivation index, BMI, smoking status, presence of rheumatoid arthritis, sickle cell disease, use of anticonvulsants, statins and iron supplements.

**Results**: Compared with people prescribed neither folic acid nor methotrexate, people prescribed folic acid supplementation had increased risk of diagnosis of COVID-19 (OR 1.51 [1.42 ; 1.61]). The prescription of methotrexate with or without folic acid was not associated with COVID-19 diagnosis (P≥0.18). People prescribed folic acid supplementation had positive association with death after a diagnosis of COVID-19 (OR 2.64 [2.15 ; 3.24]) in a fully adjusted model. The prescription of methotrexate in combination with folic acid was not associated with an increased risk for COVID-19 related death (1.07 [0.57 ; 1.98]).

**Conclusions**: We report increased risk for COVID-19 diagnosis and COVID-19-related death for people prescribed folic acid supplementation. Prescription and use of supplemental folic acid may confer increased risk of infection with SARS-CoV-2 and increased mortality with COVID-19. Our results also suggest that methotrexate might attenuate these adverse outcomes.

**Strengths and limitations of this study**

- A strength is the use of a large population-based cohort with linked data
- A strength is that the cohort was drawn from a population where food was not fortified with folic acid
- A limitation is that prescription data were single script from General Practitioners and it was not possible to assess compliance
- A limitation is that it was not possible to account for over the counter supplementation of folic acid
- A limitation is that findings cannot be generalized outside of the middle-aged (>45 years of age) white European demographic that dominates the UK Biobank cohort

**Introduction**

Folate, a B-vitamin, carries out critical roles in the transfer of one-carbon units in intermediary metabolism. Folates exist in various forms depending on the one-carbon substituent attached to the parent molecule and are involved in numerous reactions, including the synthesis of methionine from homocysteine and are also utilized in purine and pyrimidine metabolism for DNA and RNA synthesis. The oxidized form, folic acid, is added to fortified foods in the USA and over 80 other countries, including, recently, the UK[1] to prevent neural tube defect pregnancies and is used in dietary supplements to prevent or treat folate deficiency.[2] Additionally, folic acid supplementation of up to 5mg daily is often advised during pregnancy and in women of childbearing age and for other medical conditions (sickle cell anemia) [3] and during treatment with certain anticonvulsants [4].

Methotrexate, a structural analogue of folate has potent antifolate activity and is in widespread use as an antineoplastic agent and as a first-line disease-modifying antirheumatic drug (DMARD) treatment for rheumatoid arthritis (RA).[5] Folic acid (at doses commonly ranging from 1-2 mg daily) or folinic acid supplementation is often included to lower the toxicity of low-dose methotrexate therapy. [6 7]

The COVID-19 Global Rheumatology Alliance physician-reported registry has evaluated factors related to death from COVID-19 in individuals with rheumatic diseases.[8] Compared with those receiving methotrexate monotherapy, use of rituximab (OR 4.0 [95% CI 2.3 ; 7.0]), sulfasalazine (3.6 [1.7 ; 7.8]), azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and tacrolimus (2.2 [1.4 – 3.4]) or no DMARD (2.1 [1.5 – 3.0]) all had higher risks of death from COVID-19.

In order to generate purines SARS-CoV-2 post-transcriptionally remodels host folate metabolism. In an *in vitro* system using African green monkey kidney cells infected with SARS-CoV-2 intracellular glucose and folate were depleted, and this perturbation was sensitive to folate inhibitors such as methotrexate. [9] It is therefore plausible that methotrexate therapy for RA could have a beneficial effect on COVID-19

outcomes given its antifolate activity. However, since folic acid is routinely included with methotrexate to prevent methotrexate-related toxicity, such putative beneficial effect of methotrexate on viral proliferation and hence on COVID-19 outcomes may be negated by folic acid supplementation.

The aim of this study was to determine whether the use of methotrexate and folic acid prescription, together or individually, were associated with a lowered or increased risk, respectively, for COVID-19 diagnosis or mortality in a large population based-cohort.

## Participants and Methods

### Data availability

This research was conducted using the UK Biobank Resource (approval number 12611). The UK Biobank is a large resource of volunteers aged 49-86 years of age at recruitment.[10] Recruitment began in 2006 with follow-up intended for at least 30-years. SARS-CoV-2 test information, ICD-10 hospital codes, death records and general practice prescription information were obtained via the UK Biobank data portal on 13th December 2021. This information covered hospital diagnoses between 18th April 1991 and 30th September 2021, SARS-CoV-2 tests between 13th January 2020 and 18th October 2021, and death records until 12th November 2021. Illustrated in Figure 1 there were 464,306 participants, of whom 4,469 were removed owing to not having a BMI measure or Townsend index score or smoking status and a further 79,457 were removed owing to lack of prescription data. General practice prescription data from 1st of January 2019 through to 27th September 2021, available for 380,380 participants, were used to identify people prescribed methotrexate, folic acid, anticonvulsants (phenytoin, carbamazepine, phenobarbital), iron supplements (ferrous fumarate, ferrous sulfate, ferrous gluconate), and co-prescribed medications.

### Ethics approval statement

The UK Biobank was undertaken with ethical approval from the North West Multi-centre Research Ethics Committee of the UK. This study was done under this ethical approval; researchers using the UK Biobank do not require separate ethical approval. The study complies with the Declaration of Helsinki and written informed consent was obtained from all participants.

### Patient and public involvement

We did not involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

COVID-19 definitions

The criteria for COVID-19 diagnosis were defined as participants with 1) a positive SARS-CoV-2 PCR test and / or 2) ICD-10 code for confirmed COVID-19 (U07.1) or probable COVID-19 (U07.2) in hospital records, or death records. There were 26,033 cases, of whom 820 died with COVID-19. Figure 2 summarizes how cases were diagnosed.

Ethnicity, age and comorbidity data

Self-reported ethnicity was grouped into White British (British, Irish, White, any other White background), Black British (African, White and Black African, Black or Black British, Caribbean, White and Black Caribbean, Any other Black background), Asian British (Asian or Asian British, Chinese, Indian, Pakistani, Bangladeshi, White and Asian, Any other Asian background), and Other (Other ethnic group, Mixed, Any other mixed background, Do not know, Prefer not to answer). Age was calculated for 2020 from year of birth. Age groups used in the analysis were <60 years (n= 69,849), 60-69 years (n= 120,013), 70-74 years (n= 90,627) and >74 years (n= 99,891). The ICD-10 hospital codes used to determine additional comorbidity status were rheumatoid arthritis (M05), and sickle cell disease (D57). Demographic characteristics of the study population are presented in Table 1.

Statistical analysis

All association analyses were done using R v4.0.2 in RStudio 1.2.5019. Statistical model 1 was adjusted for age group (4 categories), sex, ethnicity, Townsend deprivation index, BMI, smoking status. Model 2 is Model 1 plus adjustment for the presence of rheumatoid arthritis, sickle cell disease (where daily folic acid is prescribed [3]), prescription of statins, prescription of anticonvulsants (where co-prescription of

folic acid often occurs [11]) and iron supplements (supplementary iron has been associated with poorer outcomes of infectious disease, including COVID-19 [12 13]). A $p < 0.05$ threshold indicated nominal evidence for association.

**Results**

Study population

Demographic characteristics of the study population are presented in Table 1. The proportion of those diagnosed with COVID-19 while taking methotrexate was similar to the general study population (1.14% vs 1.09%, respectively) although there was a higher proportion of methotrexate prescriptions in the group that died with COVID-19 (1.34%). There was both a higher proportion of those prescribed folic acid who were diagnosed with COVID-19 (5.99% vs 4.31% in the general population) and those prescribed folic acid in those who died with COVID-19 (15.97% vs 4.31% in the general population). Medications co-prescribed with folic acid were investigated (Table S1). Atorvastatin was co-prescribed 23.21% of the time and Simvastatin 9.49% of the time. Due to these high prescription rates and reports describing an association between statin use and reduced mortality from COVID-19 [14 15] we included statins in Model 2.

Table 1. Study population in the UK Biobank, restricted to those with data on prescriptions

| Demographic | All N= 380,380 | COVID-19 diagnosis N= 26,033 | COVID-19-related death N= 820 |
|---|---|---|---|
| <60 years of age, n(%) | 69,849 ( 18.36 ) | 7,937 ( 30.49 ) | 26 ( 3.17 ) |
| 60-70 years of age, n(%) | 120,013 ( 31.55 ) | 8,568 ( 32.91 ) | 129 ( 15.73 ) |
| 70-75 years of age, n(%) | 90,627 ( 23.83 ) | 4,569 ( 17.55 ) | 171 ( 20.85 ) |
| >75 years of age, n(%) | 99,891 ( 26.26 ) | 4,959 ( 19.05 ) | 494 ( 60.24 ) |
| Female sex, n(%) | 211,363 ( 55.57 ) | 13,802 ( 53.02 ) | 286 ( 34.88 ) |
| White British, n(%) | 357,620 ( 94.02 ) | 23,807 ( 91.45 ) | 744 ( 90.73 ) |
| Black British, n(%) | 9,826 ( 2.58 ) | 1,021 ( 3.92 ) | 36 ( 4.39 ) |
| Asian British, n(%) | 7,329 ( 1.93 ) | 732 ( 2.81 ) | 25 ( 3.05 ) |
| Other ethnicity, n(%) | 5,605 ( 1.47 ) | 473 ( 1.82 ) | 15 ( 1.83 ) |
| Prescribed methotrexate only, n(%) | 174 ( 0.05 ) | 11 ( 0.04 ) | 0 ( 0 ) |
| Prescribed folic acid only, n(%) | 12,433 ( 3.27 ) | 1,273 ( 4.89 ) | 120 ( 14.63 ) |
| Prescribed methotrexate and folic acid, n(%) | 3,952 ( 1.04 ) | 287 ( 1.10 ) | 11 ( 1.34 ) |
| Prescribed neither methotrexate nor folic acid, n(%) | 363,821 ( 95.65 ) | 24,462 ( 93.97 ) | 689 ( 84.02 ) |
| Rheumatoid arthritis, n(%) | 999 ( 0.26 ) | 97 ( 0.37 ) | 8 ( 0.98 ) |
| Sickle cell disease, n(%) | 517 ( 0.14 ) | 51 ( 0.20 ) | 2 ( 0.24 ) |
| Prescribed anticonvulsant medication, n(%) | 1,642 ( 0.43 ) | 120 ( 0.46 ) | 10 ( 1.22 ) |
| Prescribed statins, n(%) | 156,064 ( 41.03 ) | 10,398 ( 39.94 ) | 521 ( 63.54 ) |
| Prescribed iron supplements, n(%) | 18,471 ( 4.86 ) | 1,661 ( 6.38 ) | 106 ( 12.93 ) |
| BMI, mean(sd) | 27.41 ( 4.76 ) | 28.12 ( 5.03 ) | 30.21 ( 5.79 ) |
| Townsend deprivation index, mean (sd) | -1.35 ( 3.04 ) | -0.9 ( 3.18 ) | -0.28 ( 3.40 ) |
| Never smoked, n(%) | 210,993 ( 55.47 ) | 13,990 ( 53.74 ) | 321 ( 39.15 ) |
| Current smoker, n(%) | 132,222 ( 34.76 ) | 9,262 ( 35.58 ) | 384 ( 46.83 ) |
| Former smoker, n(%) | 37,165 ( 9.77 ) | 2,781 ( 10.68 ) | 115 ( 14.02 ) |

Association with a diagnosis of COVID-19

Compared with people prescribed neither folic acid nor methotrexate, individuals prescribed folic acid had significant association with diagnosis of COVID-19 in Model 1 (OR 1.60 [1.50 ; 1.70]) (Table 2). In Model 2, which included a diagnosis of RA, sickle cell disease, and prescription of anticonvulsants or statins or iron supplements, this association was not attenuated (OR 1.51 [1.42 ; 1.61]). The prescription of methotrexate without folic acid was uncommon (only 174 people) and did not show an association with COVID-19 diagnosis in either Model. The prescription of methotrexate in combination with folic acid was associated with an increased risk for a diagnosis of COVID-19 in Model 1 (1.15 [ 1.02 ; 1.30 ]) but not in Model 2 (1.09 [0.96 ; 1.23 ]) (Table 2). The risk for COVID-19 diagnosis was associated with similar magnitudes with the prescription of folic acid in men and women in Model 2 (OR 1.50 [1.37 ; 1.64] and 1.52 [1.39 ; 1.65], respectively) (Table S2). The Model 2 sex-specific associations were not statistically significant and of similar magnitudes with methotrexate and with methotrexate combined with folic acid.

Table 2. COVID-19 diagnosis in people prescribed methotrexate and / or folic acid in the UKBB, compared to people not prescribed methotrexate or folic acid

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Neither Folic acid nor Methotrexate | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 1.58 [1.49 ; 1.68 ] | <0.001 | 1.60 [1.50 ; 1.70] | <0.001 | 1.51 [1.42 ; 1.61] | <0.001 |
| Methotrexate only | 0.94 [0.51 ; 1.72] | 0.83 | 0.89 [0.48 ; 1.65] | 0.72 | 0.86 [0.47 ; 1.6] | 0.64 |
| Methotrexate and Folic acid | 1.09 [0.96 ; 1.23] | 0.18 | 1.15 [1.02 ; 1.30] | 0.021 | 1.09 [0.96 ; 1.23] | 0.18 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Association with mortality related to a COVID-19 diagnosis

In the general population, compared with people prescribed neither folic acid nor methotrexate, individuals prescribed folic acid had a significant association with mortality related to COVID-19 in Model 1 (OR 2.91 [2.38 ; 3.55]) (Table 3). In Model 2, which included a diagnosis of RA, sickle cell disease, prescription of anticonvulsants, statins and iron supplements, this association was maintained (OR 2.64 [2.15 ; 3.24]). Although there was a higher proportion of methotrexate prescriptions in the group that

died with COVID-19, there were no deaths reported in individuals diagnosed with COVID-19 who were prescribed only methotrexate (N = 11). Moreover, the prescription of methotrexate in combination with folic acid was not associated with an increased odds for death after diagnosis of COVID-19 in Model 1 (Table 3) (1.26 [ 0.70 ; 2.30 ]) or Model 2 (1.07 [0.57 ; 1.98 ]). The risk for mortality after COVID-19 diagnosis was of similar magnitude with the prescription of folic acid in both men and women in Model 2 (OR 2.59 [2.00 ; 3.36] and 2.72 [1.93 ; 3.84], respectively) (Table S3). In both men and women co-prescription of methotrexate attenuated the association.

Table 3. The association of prescription of methotrexate and folic acid with COVID-19-related death in the UK Biobank.

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither Folic acid nor Methotrexate | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 5.14 [4.23 ; 6.24] | <0.001 | 2.91 [2.38 ; 3.55] | <0.001 | 2.64 [2.15 ; 3.24] | <0.001 |
| Methotrexate and folic acid | 1.47 [0.81 ; 2.67] | 0.21 | 1.26 [0.70 ; 2.30] | 0.44 | 1.07 [0.57 ; 1.98] | 0.84 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

To account for improvements in outcome of patients with COVID-19, resulting from changes in public health measures and emergence of different SARS-CoV-2 lineages over time [16] we tested for association with death in the COVID-19-positive cohort including also a quarterly (3-monthly) categorical time variable for diagnosis of COVID-19 using Model 2 (Table S4). This revealed a similar pattern of association with death - there was association with increased risk of death in patients prescribed folic acid only (OR 1.46 [1.16 ; 1.83]) but not in the group prescribed both folic acid and methotrexate (OR 0.96 [0.50 ; 1.83]).

**Discussion**

In this population-based analysis, we report 1.5-fold increased risk for COVID-19 diagnosis and 2.6-fold increased risk for COVID-19-related death among those who had been prescribed folic acid supplementation. The prescription of methotrexate was not associated with an increased risk of diagnosis of COVID-19 and we were not able to make an estimate for COVID-19-related death in the small sample of those prescribed methotrexate only. Notably, those prescribed methotrexate and folic acid did not have an increased risk for COVID-19 diagnosis or associated death, indicating that methotrexate might attenuate an increased risk for COVID-19 diagnosis and related-death conferred by folic acid.

In the context of SARS-Cov-2 infection it is established that hijacking of cellular metabolic pathways is important for viral replication.[17] Zhang et al described that SARS-CoV-2 remodels host folate and one-carbon metabolism at the post-transcriptional level to support de novo purine synthesis, bypassing viral shutoff of host translation. [9] This suggests that viral replication could be sensitive to folate inhibitors, such as methotrexate. Intracellular glucose and folate are depleted in SARS-CoV-2-infected cells, and viral replication is exquisitely sensitive *in vitro* to inhibitors of folate and one carbon metabolism, notably methotrexate.[9] Stegmann et al, based on cell culture experiments, reported that methotrexate alone or in combination with remdesivir limits the replication of SARS-CoV-2.[18] With the caveat that our study is observational epidemiology and causality cannot be inferred, our study does support the possibility that external folate supply facilitates the production of large amounts of virus, contributing to clinical infection and mortality. Our study also supports the notion that SARS-CoV-2 replication is enhanced by folate supply based on our finding that co-prescription of an antifolate (methotrexate) can ameliorate the possibly adverse effect of supplementation with folic acid on COVID-19 outcomes.

There is also evidence that inadequate folate status may be harmful in the context of host resistance to infection with SARS-CoV-2. In addition to the well-recognized complication of anemia, folate deficiency has other detrimental health effects, including suppression of immune function.[19] Additional support for the concept that adequate folate status is important in COVID-19 outcomes is provided by the observation that folate deficiency was associated with poorer outcomes in a cohort of COVID-19 patients.[20] It is important to note that it is possible that in the study by Itelman et al,[20] if increased folate levels were causal of COVID-19 diagnosis and poor outcomes, that the association with lower folate levels could have been caused by selection (collider) bias.[21] Vitamin B12 deficiency has also been proposed as a factor related to poor COVID-19 outcomes, presumed to be through the induction of functional folate deficiency.[22] A drug-protein structure interaction analysis raises the possibility that folate blocks the 3CL hydrolase enzyme, which may affect viral entry and replication.[23] It is therefore possible that both inadequate and excessive amounts of folate may be detrimental to host resistance to SARS-CoV-2 infection and that there may be an optimal range of physiological folate status related to host-resistance to COVID-19 infection and severity.

Data from the COVID-19 Global Rheumatology Alliance describes that a number of immunomodulatory drugs used in rheumatology are associated with an increased risk of infection and death compared with methotrexate. [8] Being on no DMARD therapy was associated with an increased risk of death with COVID-19 (OR 2.11 [1.48 ; 3.01]), which could be interpreted as either a protective effect of methotrexate or an increased risk for death associated with poor rheumatic disease control. The authors of the study additionally noted that people not on DMARD therapy had increased use of glucocorticoids meaning that confounding by indication cannot be ruled out as an explanation.[24] Methotrexate was also associated with lower odds for death when compared with sulfasalazine, other immunosuppressants and rituximab. In no case was methotrexate associated with an increased risk for death. The COVID-19 Global Rheumatology Alliance study did not explore the effect of folic acid supplementation in the

setting of methotrexate, although it is it is highly likely that almost all patients on methotrexate also were receiving folic acid supplementation. Considering the widespread use of folic acid supplements and proposals to abandon entirely tolerable upper intake levels for folic acid [25] it would be prudent to monitor the effect of increased folic acid intake at a population level on COVID-19 morbidity and mortality, particularly at the upper end of folic acid intake.

A number of limitations of our analysis are important to note. One, given the small size of the methotrexate-only group and that there were no deaths related to COVID-19 in this group we could not test a beneficial effect on mortality of methotrexate in isolation. It is uncommon to find patients with methotrexate prescribed without supplemental folic acid as this is the standard of care. Second, over the time period of this study (March 2020 – November 2021), COVID-19 outcomes (i.e. death) will have been influenced by the development of clinical treatments including antiviral drugs and monoclonal antibodies, changes to public health measures and the appearance of new COVID-19 strains [16] . We were unable to account for these factors in the population-based analysis however we attempted to account for this in the analysis within the COVID-19-positive group by including a time variable (Table S4). Third, findings are not necessarily generalizable outside of the middle-aged (>45 years of age) white European cohort that dominates the UK Biobank. Fourth, the full extent of SARS-CoV-2 infection is not known in the UK population due to incomplete testing rates early in the pandemic. Fifth, prescription data were single script from General Practitioners only, and it was not possible to ascertain compliance or whether participants were taking the prescribed medication during the COVID-19 pandemic although we attempted to account for this by only using prescription information from 2019 and 2020. Sixth, although we included RA in Model 2, we were unable to account for any potential effect of disease activity in RA in people prescribed folic acid. Disease activity negatively impacts death from COVID-19 outcomes.[8] Finally, while it is a strength of our study that mandatory fortification of the UK diet with folic acid had not been introduced during the period of our study and thus did not confound our

analysis, we were unable to account for the lower-dose over the counter folic acid supplementation available in the UK (400 micrograms being the most common formulation for over the counter tablets) because there were no self-report information in the UK Biobank dataset on the use of folic acid supplementation.

In conclusion, and despite the limitations of our study enumerated above, our data support the hypothesis that increased folate resulting from folic acid prescription could contribute to a higher probability of contracting clinically-detectable infection with SARS-CoV2 and to an increase in the risk of death following the infection. The study population was drawn from the >45 year old segment of the UK population and is predominantly of white European ethnicity, therefore our findings have reduced generalizability to younger people, to other ethnic groups and to other countries. Nevertheless, our findings justify future studies on the influence of folic acid supplementation on COVID-19 outcomes, particularly in pregnant women and people on anticonvulsants requiring supplementary folic acid.

As a final comment, we point out that attention is currently being directed toward establishing whether excessive intake of folate, particularly in the form of folic acid, may have undesirable and potentially deleterious effects.[26] The possibility that susceptibility to COVID-19 infection and its serious and even fatal complications may be affected by folic acid intake and folate status should be thoroughly investigated.

**Acknowledgements**

This research was conducted using the UK Biobank Resource under Application Number 12611. We sincerely thank all participants.

**Declarations of interest**

PCR reports personal fees from Abbvie, Atom Biosciences, Eli Lilly, Gilead, Janssen, Novartis, UCB, Roche, Pfizer; meeting attendance support from BMS, Pfizer and UCB Pharma and grant funding from Janssen, Novartis, Pfizer and UCB Pharma, all outside the submitted work. AG reports personal fees from SOBI, Selecta and honoraria from UptoDate, Inc. outside the submitted work. All other authors have no declarations of interest.

**Author contributions**

All authors substantially contributed to study conception and design, to acquisition and analysis of data and interpretation of results. All authors contributed to drafting the article and critical revision and all authors approved the final version. RKT and TRM directly accessed and verified the underlying data reported in the manuscript.

**Data sharing**

All data utilised in this study were accessed from the publicly available UK Biobank Resource under Application Number 12611. These data cannot be shared with other investigators.

**Figure legends**

**Figure 1 Flow schematic of study design**

**Figure 2 Data sources of COVID-19-diagnosed individuals**

Of the 26,033 COVID-19-diagnosed individuals, 25,300 were identified from positive SARS-Cov2 test results (21,705 unique to this group), 4,170 identified from hospital records (650 unique to this group), and 820 identified from death records (51 unique to this group). 217 diagnosed after 13th October 2021 (30 days before the last recorded death) were removed from the case only analysis in Table S4 given the unknown outcome.

**Funding Statement**

There was no funding to report for this research study

REFERENCES


1. Haggarty P. UK introduces folic acid fortification of flour to prevent neural tube defects. *The Lancet* 2021;398(10307):1199-201. doi: https://doi.org/10.1016/S0140-6736(21)02134-6
2. Department of Health and Social Care UG. [Available from: https://www.gov.uk/government/news/folic-acid-added-to-flour-to-prevent-spinal-conditions-in-babies accessed 1/11 2022.
3. Dixit R, Nettem S, Madan SS, et al. Folate supplementation in people with sickle cell disease. *Cochrane Database Syst Rev* 2018;3:CD011130. doi: 10.1002/14651858.CD011130.pub3 [published Online First: 2018/03/17]
4. Linnebank M, Moskau S, Semmler A, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol* 2011;69(2):352-9. doi: 10.1002/ana.22229 [published Online First: 2011/01/20]
5. Fraenkel L, Bathon JM, England BR, et al. 2021 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol* 2021;73(7):1108-23. doi: 10.1002/art.41752 [published Online First: 2021/06/09]
6. Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;121(11):833-41. doi: 10.7326/0003-4819-121-11-199412010-00002 [published Online First: 1994/12/01]
7. Morgan SL, Baggott JE, Vaughn WH, et al. The effect of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 1990;33(1):9-18. doi: 10.1002/art.1780330102 [published Online First: 1990/01/01]
8. Strangfeld A, Schäfer M, Gianfrancesco MA, et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Annals of the Rheumatic Diseases* 2021;80(7):930-42. doi: 10.1136/annrheumdis-2020-219498
9. Zhang Y, Guo R, Kim SH, et al. SARS-CoV-2 hijacks folate and one-carbon metabolism for viral replication. *Nature Communications* 2021;12(1):1676. doi: 10.1038/s41467-021-21903-z
10. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12(3):e1001779. doi: 10.1371/journal.pmed.1001779 [published Online First: 2015/04/01]
11. Moore JL. The significance of folic acid for epilepsy patients. *Epilepsy Behav* 2005;7(2):172-81. doi: 10.1016/j.yebeh.2005.05.020 [published Online First: 2005/08/02]
12. Girelli D, Marchi G, Busti F, et al. Iron metabolism in infections: Focus on COVID-19. *Semin Hematol* 2021;58(3):182-87. doi: 10.1053/j.seminhematol.2021.07.001 [published Online First: 2021/08/15]
13. Gonciarz RL, Renslo AR. Emerging role of ferrous iron in bacterial growth and host-pathogen interaction: New tools for chemical (micro)biology and antibacterial therapy. *Curr Opin Chem Biol* 2021;61:170-78. doi: 10.1016/j.cbpa.2021.01.015 [published Online First: 2021/03/15]
14. Diaz-Arocutipa C, Melgar-Talavera B, Alvarado-Yarasca A, et al. Statins reduce mortality in patients with COVID-19: an updated meta-analysis of 147 824 patients. *Int J Infect Dis* 2021;110:374-81. doi: 10.1016/j.ijid.2021.08.004 [published Online First: 2021/08/11]
15. Kollias A, Kyriakoulis KG, Kyriakoulis IG, et al. Statin use and mortality in COVID-19 patients: Updated systematic review and meta-analysis. *Atherosclerosis* 2021;330:114-21. doi: 10.1016/j.atherosclerosis.2021.06.911 [published Online First: 2021/07/11]

16. Davies NG, Jarvis CI, Group CC-W, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature* 2021;593(7858):270-74. doi: 10.1038/s41586-021-03426-1 [published Online First: 2021/03/17]
17. Thaker SK, Ch'ng J, Christofk HR. Viral hijacking of cellular metabolism. *BMC Biology* 2019;17(1):59. doi: 10.1186/s12915-019-0678-9
18. Stegmann KM, Dickmanns A, Gerber S, et al. The folate antagonist methotrexate diminishes replication of the coronavirus SARS-CoV-2 and enhances the antiviral efficacy of remdesivir in cell culture models. *Virus Res* 2021;302:198469. doi: 10.1016/j.virusres.2021.198469 [published Online First: 2021/06/07]
19. Green R, Miller JW. Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations of dysfunctional folate status. *Semin Hematol* 1999;36(1):47-64. [published Online First: 1999/02/04]
20. Itelman E, Wasserstrum Y, Segev A, et al. Clinical Characterization of 162 COVID-19 patients in Israel: Preliminary Report from a Large Tertiary Center. *Isr Med Assoc J* 2020;22(5):271-74. [published Online First: 2020/05/08]
21. Choi HK, Nguyen US, Niu J, et al. Selection bias in rheumatic disease research. *Nat Rev Rheumatol* 2014;10(7):403-12. doi: 10.1038/nrrheum.2014.36 [published Online First: 2014/04/02]
22. Wee AKH. COVID-19's toll on the elderly and those with diabetes mellitus - Is vitamin B12 deficiency an accomplice? *Med Hypotheses* 2021;146:110374. doi: 10.1016/j.mehy.2020.110374 [published Online First: 2020/12/02]
23. Serseg T, Benarous K, Yousfi M. Hispidin and Lepidine E: Two Natural Compounds and Folic Acid as Potential Inhibitors of 2019-novel Coronavirus Main Protease (2019- nCoVM(pro)), Molecular Docking and SAR Study. *Curr Comput Aided Drug Des* 2021;17(3):469-79. doi: 10.2174/1573409916666200422075440 [published Online First: 2020/04/24]
24. Schafer M, Strangfeld A, Hyrich KL, et al. Response to: 'Correspondence on 'Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician reported registry'' by Mulhearn et al. *Ann Rheum Dis* 2021 doi: 10.1136/annrheumdis-2021-220134 [published Online First: 2021/03/03]
25. Wald NJ, Morris JK, Blakemore C. Public health failure in the prevention of neural tube defects: time to abandon the tolerable upper intake level of folate. *Public Health Rev* 2018;39:2. doi: 10.1186/s40985-018-0079-6 [published Online First: 2018/02/17]
26. Maruvada P, Stover PJ, Mason JB, et al. Knowledge gaps in understanding the metabolic and clinical effects of excess folates/folic acid: a summary, and perspectives, from an NIH workshop. *Am J Clin Nutr* 2020;112(5):1390-403. doi: 10.1093/ajcn/nqaa259 [published Online First: 2020/10/07]

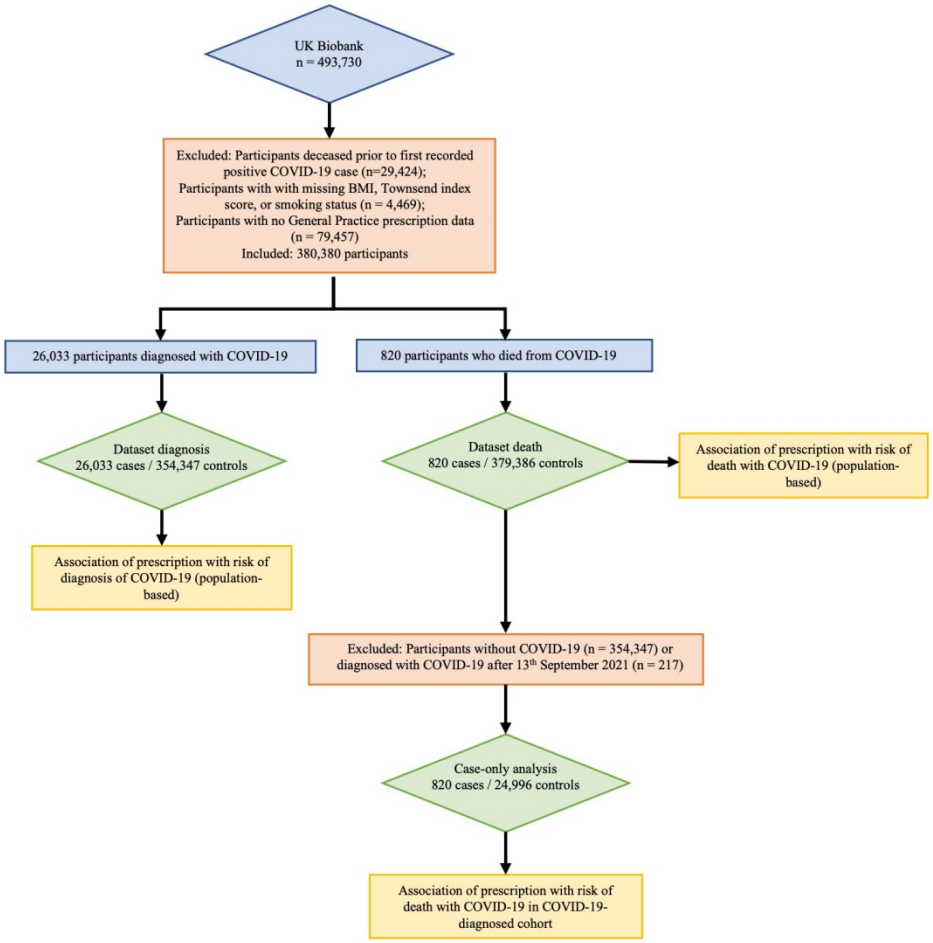


Figure 1 Flow schematic of study design

181x190mm (225 x 225 DPI)

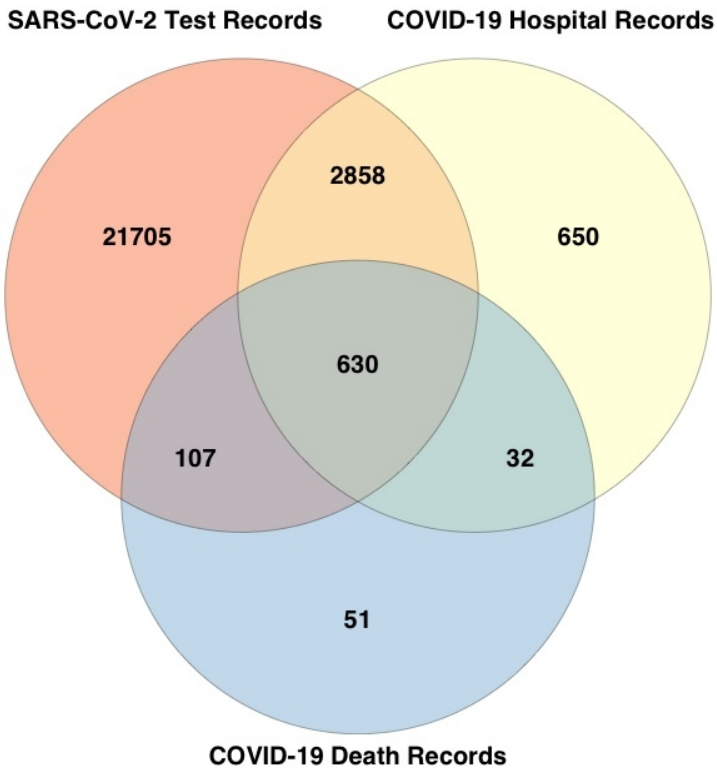


Figure 2 Data sources of COVID-19-diagnosed individuals. Of the 26,033 COVID-19-diagnosed individuals, 25,300 were identified from positive SARS-Cov2 test results (21,705 unique to this group), 4,170 identified from hospital records (650 unique to this group), and 820 identified from death records (51 unique to this group). 217 diagnosed after 13th October 2021 (30 days before the last recorded death) were removed from the case only analysis in Table S4 given the unknown outcome.

211x211mm (96 x 96 DPI)

Table S1. Medications co-prescribed with folic acid on >5% of occasions

| Medication/Supplement | Number of prescriptions* | Percentage of folic acid prescriptions |
|---|---|---|
| Folic acid | 177,918 | 100.00 |
| Methotrexate | 45,697 | 25.68 |
| Atorvastatin | 41,296 | 23.21 |
| Lansoprazole | 35,611 | 20.02 |
| Omeprazole | 32,909 | 18.50 |
| Bisoprolol | 26,445 | 14.86 |
| Levothyroxine | 25,482 | 14.32 |
| Ramipril | 21,990 | 12.36 |
| Aspirin | 21,696 | 12.19 |
| Paracetamol | 20,830 | 11.71 |
| Amlodipine | 18,354 | 10.32 |
| Simvastatin | 16,878 | 9.49 |
| Amitriptyline | 13,573 | 7.63 |
| Furosemide | 12,983 | 7.30 |
| Metformin | 11,460 | 6.44 |
| Hydroxychloroquine | 11,038 | 6.20 |
| Clopidogrel | 10,918 | 6.14 |
| Prednisolone | 10,475 | 5.89 |
| Alendronic acid | 10,198 | 5.73 |

*General practice prescription data from 1st of January 2019 through to 27th September 2021

Table S2. Sex-specific associations between prescription of methotrexate, folic acid, and the combination of both with the risk of COVID-19 diagnosis

| | COVID-19 diagnosis - Male | | | | | | COVID-19 diagnosis -Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | | *Model 1* | | *Model 2* | | *Unadjusted* | | *Model 1* | | *Model 2* | |
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | OR, [95% CI] | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 1.52 [ 1.39 ; 1.66 ] | <0.001 | 1.57 [ 1.44 ; 1.72 ] | <0.001 | 1.50 [ 1.37 ; 1.64 ] | <0.001 | 1.64 [ 1.51 ; 1.77 ] | <0.001 | 1.61 [ 1.48 ; 1.75 ] | <0.001 | 1.52 [ 1.39 ; 1.65 ] | <0.001 |
| Methotrexate only | 0.96 [ 0.39 ; 2.38 ] | 0.93 | 0.93 [ 0.37 ; 2.32 ] | 0.88 | 0.92 [ 0.37 ; 2.30 ] | 0.86 | 0.92 [ 0.4 ; 2.11 ] | 0.85 | 0.87 [ 0.38 ; 1.99 ] | 0.74 | 0.83 [ 0.36 ; 1.90 ] | 0.65 |
| Methotrexate and folic acid | 1.08 [ 0.89 ; 1.31 ] | 0.45 | 1.15 [ 0.94 ; 1.40 ] | 0.17 | 1.11 [ 0.91 ; 1.36 ] | 0.29 | 1.11 [ 0.95 ; 1.29 ] | 0.19 | 1.16 [ 0.99 ; 1.36 ] | 0.060 | 1.07 [ 0.91 ; 1.26 ] | 0.38 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Table S3. Sex-specific associations between prescription of methotrexate, folic acid, and the combination of both with the risk of COVID-19 related mortality.

| | COVID-19 related death - Male | | | | | | COVID-19 related death - Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | | *Model 1* | | *Model 2* | | *Unadjusted* | | *Model 1* | | *Model 2* | |
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | OR, [95% CI] | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 4.73 [3.71; 6.04] | <0.001 | 2.83 [2.20; 3.64 ] | <0.001 | 2.59 [2.00; 3.36] | <0.001 | 5.67 [4.10; 7.82 ] | <0.001 | 3.07 [2.20; 4.28] | <0.001 | 2.72 [1.93; 3.84] | <0.001 |
| Methotrexate and folic acid | 2.00 [0.99; 4.03] | 0.053 | 1.62 [0.80; 3.26] | 0.18 | 1.54 [0.75; 3.15] | 0.24 | 1.01 [0.32; 3.16] | 0.99 | 0.80 [0.25; 2.50] | 0.70 | 0.56 [0.17; 1.84] | 0.34 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status
Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Table S4. Risk of death related to COVID-19 in the COVID-19-positive group

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 3.58 [ 2.93 ; 4.39 ] | <0.001 | 1.82 [ 1.47 ; 2.26 ] | <0.001 | 1.46 [ 1.16 ; 1.83 ] | 0.0011 |
| Methotrexate and folic acid | 1.37 [ 0.75 ; 2.52 ] | 0.31 | 1.03 [ 0.55 ; 1.91 ] | 0.94 | 0.96 [ 0.50 ; 1.83 ] | 0.90 |

Model 2 additionally adjusted by quarterly categorical time variable.

A total of 25,816 COVID-19-positive cases were analyzed. 217 COVID-19-positive cases who were diagnosed after 13th September 2021 (28 days before the last recorded death) were removed from the cohort given the unknown outcome in these individuals

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 3 | 'A population-based cohort' in third line of the abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 3 | Throughout abstract |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 6-7 | All text in Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 7 | Last sentence of Introduction. |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | 3,7 | Refer items 1a, 1b and 3 above. |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-9 | Throughout the methods |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 8-9 (case-control) | This information is in the Methods sections 'COVID-19 definitions' and 'Prescription data' |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | | Matching was not done in this study. |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 8 | This information is in the Methods section 'Ethnicity, age and comorbidity data'. |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 8-9 | This information is in the Methods sections 'Gout and COVID-19 definitions and case- |

| | | | | |
|---|---|---|---|---|
| | | | | control datasets' and 'Ethnicity, age and comorbidity data'. |
| Bias | 9 | Describe any efforts to address potential sources of bias | Table S4 | This analysis including a categorical time variable was done to account for improvements in treatment over time |
| Study size | 10 | Explain how the study size was arrived at | | The study size comprised all available data from the UK Biobank. |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | | Quantitative variables were BMI, age and Townsend deprivation index. These were all included as potential confounding variables. Age groupings described in the final paragraph on page 6. |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 9 | Refer to Methods section 'Statistical analysis'. |
| | | (*b*) Describe any methods used to examine subgroups and interactions | | The COVID-19 case-only analysis was one subgroup (Table S4). Additional subgroups were men and women. |
| | | (*c*) Explain how missing data were addressed | 8 | Paragraph 1: "there were 464,306 participants, of whom 4,469 were removed owing to not having a BMI measure or Townsend index score or smoking status and a further 112,466 were removed owing to lack of prescription data." |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | | Not applicable |
| | | (*e*) Describe any sensitivity analyses | | No sensitivity analyses were done. |
| **Results** | | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | | Refer Figure 1. |
| | | (b) Give reasons for non-participation at each stage | 8 | Refer paragraph 1. |
| | | (c) Consider use of a flow diagram | | Figure 1 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 18-19 | Table 1 |

| | | | | |
|---|---|---|---|---|
| | | (b) Indicate number of participants with missing data for each variable of interest | | Not applicable. Participants with missing data were excluded. |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | | Not applicable |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | | Not applicable. |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | 18-19 | Table 1 |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 9-10,20-21 | Unadjusted estimates are provided (Tables 2,3). The reason for inclusion of potential confounders in Model 2 is explained in last paragraph pg 9 and first paragraph pg 10. |
| | | (*b*) Report category boundaries when continuous variables were categorized | | This was done for age: "Age was calculated for 2020 from year of birth. Age groups used in the analysis were <60 years (n= 57,618), 60-69 years (n= 107,140), 70-74 years (n= 85,926) and >74 years (n= 96,687)." |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | | Not applicable |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 23-24,26 | Analyses stratified by sex and vaccination status reported in Tables S2, S3, S5. |
| **Discussion** | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 | First paragraph: "In this population-based analysis, we report 1.7-fold increased risk for COVID-19 diagnosis and 3.2-fold increased risk for COVID-19-related death among those having been prescribed folic acid supplementation. The prescription of methotrexate was not associated with an increased risk of diagnosis of COVID-19" |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15-16 | Eight specific limitations are discussed |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-14 | First three paragraphs of Discussion |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 | Paragraph 2: "findings are not necessarily generalizable outside of the middle-aged (>45 years of age) white European cohort that dominates the UK Biobank." |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 7 | Not applicable |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

# FOLIC ACID AND METHOTREXATE USE AND THEIR ASSOCIATION WITH COVID-19 DIAGNOSIS AND MORTALITY: AN ANALYSIS FROM THE UK BIOBANK



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2022-062945.R1 |
| Article Type: | Original research |
| Date Submitted by the Author: | 24-Jun-2022 |
| Complete List of Authors: | Topless, Ruth; University of Otago<br>Green, Ralph; University of California Davis at Sacramento, Departments of Pathology and Medicine<br>Morgan, Sarah; The University of Alabama at Birmingham<br>Robinson, Philip ; University of Queensland Faculty of Medicine and Biomedical Sciences<br>Merriman, Tony; University of Otago, School of Medical Sciences<br>Gaffo, Angelo; The University of Alabama at Birmingham; Birmingham VA Medical Center |
| Primary Subject Heading: | Epidemiology |
| Secondary Subject Heading: | Rheumatology, Infectious diseases |
| Keywords: | COVID-19, Epidemiology < INFECTIOUS DISEASES, Rheumatology < INTERNAL MEDICINE |
| | |

*I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.*

*The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.*

*Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.*

# FOLIC ACID AND METHOTREXATE USE AND THEIR ASSOCIATION WITH COVID-19 DIAGNOSIS AND MORTALITY: AN ANALYSIS FROM THE UK BIOBANK

Ruth K Topless BSc [1], Ralph Green MD PhD FRCPath[2] , Sarah L. Morgan MD RD[3], Philip C Robinson MBChB PhD [4], Tony R Merriman PhD[1,3] , Angelo L. Gaffo MD MsPH FACP [3,5]

[1]Department of Biochemistry, University of Otago, Dunedin, New Zealand.

[2]Departments of Pathology and Medicine, University of California Davis at Sacramento, Sacramento, California, USA.

[3]Division of Clinical Immunology and Rheumatology, The University of Alabama at Birmingham, Birmingham, Alabama, USA.

[4]School of Clinical Medicine, University of Queensland, Brisbane, Australia

[5]Birmingham VA Medical Center. Birmingham, Alabama, USA

**Word count in this version: 3797**

**Corresponding Authors and reprint requests:**

Angelo Gaffo

SHEL 306

1720 2nd Ave South

Birmingham, AL 35294.

USA

Phone: +1-205-9348909

Fax: +1-205-9966788

agaffo@uabmc.edu

Ralph Green

4400 V St.

Sacramento, CA 95817

Phone: +1-916-7348078

Fax: +1-916 – 7340299

rgreen@ucdavis.edu

**Abstract**

**Objective**: to determine if methotrexate or folic acid prescription were associated with differential risk for COVID-19 diagnosis or mortality.

**Design**: Case-control analysis.

**Setting**: the population-based UK Biobank (UKBB) cohort.

**Participants**: Data from 380,380 UKBB participants with general practice prescription data for 2019 to 2021. Updated medical information was retrieved on the 13th December 2021.

**Primary and secondary outcome measures**: The outcomes of COVID-19 diagnosis and COVID-19 related mortality were analyzed by multivariable logistic regression. Exposures evaluated were prescription of folic acid and/or methotrexate. Criteria for COVID-19 diagnosis were 1) a positive SARS-CoV-2 test or 2) ICD-10 code for confirmed COVID-19 (U07.1) or probable COVID-19 (U07.2) in hospital records, or death records. By these criteria 26,003 individuals were identified with COVID-19 of whom 820 were known to have died from COVID-19. Logistic regression statistical models were adjusted for age sex, ethnicity, Townsend deprivation index, BMI, smoking status, presence of rheumatoid arthritis, sickle cell disease, use of anticonvulsants, statins and iron supplements.

**Results**: Compared with people prescribed neither folic acid nor methotrexate, people prescribed folic acid supplementation had increased risk of diagnosis of COVID-19 (OR 1.51 [1.42 ; 1.61]). The prescription of methotrexate with or without folic acid was not associated with COVID-19 diagnosis (P≥0.18). People prescribed folic acid supplementation had positive association with death after a diagnosis of COVID-19 (OR 2.64 [2.15 ; 3.24]) in a fully adjusted model. The prescription of methotrexate in combination with folic acid was not associated with an increased risk for COVID-19 related death (1.07 [0.57 ; 1.98]).

**Conclusions**: We report increased risk for COVID-19 diagnosis and COVID-19-related death for people prescribed folic acid supplementation. Prescription and use of supplemental folic acid may confer increased risk of infection with SARS-CoV-2 and increased mortality with COVID-19. Our results also suggest that methotrexate might attenuate these adverse outcomes.

**Strengths and limitations of this study**

- A strength is the use of a large population-based cohort with linked data
- A strength is that the cohort was drawn from a population where food was not fortified with folic acid
- A limitation is that prescription data were single script from General Practitioners and it was not possible to assess compliance
- A limitation is that it was not possible to account for over the counter supplementation of folic acid
- A limitation is that findings cannot be generalized outside of the middle-aged (>45 years of age) white European demographic that dominates the UK Biobank cohort

**Introduction**

Folate, a B-vitamin, carries out critical roles in the transfer of one-carbon units in intermediary metabolism. Folates exist in various forms depending on the one-carbon substituent attached to the parent molecule and are involved in numerous reactions, including the synthesis of methionine from homocysteine and are also utilized in purine and pyrimidine metabolism for DNA and RNA synthesis. The oxidized form, folic acid, is presently added to fortified foods in the USA and over 80 other countries. Recently, a decision has been taken in the UK to introduce fortification to prevent neural tube defect pregnancies. Folic acid also is used in dietary supplements to prevent or treat folate deficiency.[1] Additionally, folic acid supplementation of up to 5mg daily is often advised during pregnancy and in women of childbearing age and for other medical conditions (sickle cell anemia) [2] and during treatment with certain anticonvulsants [3].

Methotrexate, a structural analogue of folate has potent antifolate activity and is in widespread use as an antineoplastic agent and as a first-line disease-modifying antirheumatic drug (DMARD) treatment for rheumatoid arthritis (RA).[4] Folic acid (at doses commonly ranging from 1-2 mg daily) or folinic acid supplementation is often included to lower the toxicity of low-dose methotrexate therapy. [5 6]

The COVID-19 Global Rheumatology Alliance physician-reported registry has evaluated factors related to death from COVID-19 in individuals with rheumatic diseases.[7] Compared with those receiving methotrexate monotherapy, use of rituximab (OR 4.0 [95% CI 2.3 ; 7.0]), sulfasalazine (3.6 [1.7 ; 7.8]), azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and tacrolimus (2.2 [1.4 – 3.4]) or no DMARD (2.1 [1.5 – 3.0]) all had higher risks of death from COVID-19.

In order to generate purines SARS-CoV-2 post-transcriptionally remodels host folate metabolism. In an *in vitro* system using African green monkey kidney cells infected with SARS-CoV-2 intracellular glucose and folate were depleted, and this perturbation was sensitive to folate inhibitors such as methotrexate.

[8] It is therefore plausible that methotrexate therapy for RA could have a beneficial effect on COVID-19 outcomes given its antifolate activity. However, since folic acid is routinely included with methotrexate to prevent methotrexate-related toxicity, such putative beneficial effect of methotrexate on viral proliferation and hence on COVID-19 outcomes may be negated by folic acid supplementation.

The aim of this study was to determine whether the use of methotrexate and folic acid prescription, together or individually, were associated with a lowered or increased risk, respectively, for COVID-19 diagnosis or mortality in a large population based-cohort.

## Participants and Methods

### Data availability

This research was conducted using the UK Biobank Resource (approval number 12611).[9] The UK Biobank is a large resource of volunteers aged 49-86 years of age at recruitment.[10] Recruitment began in 2006 with follow-up intended for at least 30-years. SARS-CoV-2 test information, ICD-10 hospital codes, death records and general practice prescription information were obtained via the UK Biobank data portal on 13th December 2021. This information covered hospital diagnoses between 18th April 1991 and 30th September 2021, SARS-CoV-2 tests between 13th January 2020 and 18th October 2021, and death records until 12th November 2021. Illustrated in Figure 1 there were 464,306 participants, of whom 4,469 were removed owing to not having a BMI measure or Townsend index score or smoking status and a further 79,457 were removed owing to lack of prescription data. General practice prescription data from 1st of January 2019 through to 27th September 2021, available for 380,380 participants, were used to identify people prescribed methotrexate, folic acid, anticonvulsants (phenytoin, carbamazepine, phenobarbital), iron supplements (ferrous fumarate, ferrous sulfate, ferrous gluconate), and co-prescribed medications.

### Ethics approval statement

The UK Biobank was undertaken with ethical approval from the North West Multi-centre Research Ethics Committee of the UK. This study was done under this ethical approval; researchers using the UK Biobank do not require separate ethical approval. The study complies with the Declaration of Helsinki and written informed consent was obtained from all participants.

### Patient and public involvement

We did not involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

COVID-19 definitions

The criteria for COVID-19 diagnosis were defined as participants with 1) a positive SARS-CoV-2 PCR test and / or 2) ICD-10 code for confirmed COVID-19 (U07.1) or probable COVID-19 (U07.2) in hospital records, or death records. There were 26,033 cases, of whom 820 died with COVID-19. Figure 2 summarizes how cases were diagnosed.

Ethnicity, age and comorbidity data

Self-reported ethnicity was grouped into White British (British, Irish, White, any other White background), Black British (African, White and Black African, Black or Black British, Caribbean, White and Black Caribbean, Any other Black background), Asian British (Asian or Asian British, Chinese, Indian, Pakistani, Bangladeshi, White and Asian, Any other Asian background), and Other (Other ethnic group, Mixed, Any other mixed background, Do not know, Prefer not to answer). Age was calculated for 2020 from year of birth. Age groups used in the analysis were <60 years (n= 69,849), 60-69 years (n= 120,013), 70-74 years (n= 90,627) and >74 years (n= 99,891). The ICD-10 hospital codes used to determine additional comorbidity status were rheumatoid arthritis (M05), and sickle cell disease (D57). Demographic characteristics of the study population are presented in Table 1.

Statistical analysis

All association analyses were done using R v4.0.2 in RStudio 1.2.5019. Statistical model 1 was adjusted for age group (4 categories), sex, ethnicity, Townsend deprivation index, BMI, smoking status. Model 2 is Model 1 plus adjustment for the presence of rheumatoid arthritis, sickle cell disease (where daily folic acid is prescribed [2]), prescription of statins, prescription of anticonvulsants (where co-prescription of

folic acid often occurs [11]) and iron supplements (supplementary iron has been associated with poorer outcomes of infectious disease, including COVID-19 [12 13]). For methotrexate and folate use, a single variable with 4 levels was used for statistical modeling (no methotrexate or folate, methotrexate only, folate only, methotrexate and folate). Sex-stratified analyses were done using the same approach to explore any differential association with COVID-19 diagnosis or associated mortality. A $p < 0.05$ threshold indicated nominal evidence for association.

**Results**

Study population

Demographic characteristics of the study population are presented in Table 1. The proportion of those diagnosed with COVID-19 while taking methotrexate was similar to the general study population (1.14% vs 1.09%, respectively) although there was a higher proportion of methotrexate prescriptions in the group that died with COVID-19 (1.34%). There was both a higher proportion of those prescribed folic acid who were diagnosed with COVID-19 (5.99% vs 4.31% in the general population) and those prescribed folic acid in those who died with COVID-19 (15.97% vs 4.31% in the general population). Medications co-prescribed with folic acid were investigated (Table S1). Atorvastatin was co-prescribed 23.21% of the time and Simvastatin 9.49% of the time. Due to these high prescription rates and reports describing an association between statin use and reduced mortality from COVID-19 [14 15] we included statins in Model 2.

Table 1. Study population in the UK Biobank, restricted to those with data on prescriptions

| Demographic | All N= 380,380 | COVID-19 diagnosis N= 26,033 | No COVID-19 diagnosis N= 354,347 | COVID-19-related death N= 820 | No COVID-19-related death N= 379,560 |
|---|---|---|---|---|---|
| <60 years of age, n(%) | 69,849 ( 18.36 ) | 7,937 ( 30.49 ) | 61,912 ( 17.47 ) | 26 ( 3.17 ) | 69,823 ( 18.4 ) |
| 60-70 years of age, n(%) | 120,013 ( 31.55 ) | 8,568 ( 32.91 ) | 111,445 ( 31.45 ) | 129 ( 15.73 ) | 119,884 ( 31.58 ) |
| 70-75 years of age, n(%) | 90,627 ( 23.83 ) | 4,569 ( 17.55 ) | 86,058 ( 24.29 ) | 171 ( 20.85 ) | 90,456 ( 23.83 ) |
| >75 years of age, n(%) | 99,891 ( 26.26 ) | 4,959 ( 19.05 ) | 94,932 ( 26.79 ) | 494 ( 60.24 ) | 99,397 ( 26.19 ) |
| Female sex, n(%) | 211,363 ( 55.57 ) | 13,802 ( 53.02 ) | 197,561 ( 55.75 ) | 286 ( 34.88 ) | 211,077 ( 55.61 ) |
| White British, n(%) | 357,620 ( 94.02 ) | 23,807 ( 91.45 ) | 333,813 ( 94.21 ) | 744 ( 90.73 ) | 356,876 ( 94.02 ) |
| Black British, n(%) | 9,826 ( 2.58 ) | 1,021 ( 3.92 ) | 8,805 ( 2.48 ) | 36 ( 4.39 ) | 9,790 ( 2.58 ) |
| Asian British, n(%) | 7,329 ( 1.93 ) | 732 ( 2.81 ) | 6,597 ( 1.86 ) | 25 ( 3.05 ) | 7,304 ( 1.92 ) |
| Other ethnicity, n(%) | 5,605 ( 1.47 ) | 473 ( 1.82 ) | 5,132 ( 1.45 ) | 15 ( 1.83 ) | 5,590 ( 1.47 ) |
| Prescribed methotrexate only, n(%) | 174 ( 0.05 ) | 11 ( 0.04 ) | 163 ( 0.05 ) | 0 ( 0 ) | 174 ( 0.05 ) |
| Prescribed folic acid only, n(%) | 12,433 ( 3.27 ) | 1,273 ( 4.89 ) | 11,160 ( 3.15 ) | 120 ( 14.63 ) | 12,313 ( 3.24 ) |
| Prescribed methotrexate and folic acid, n(%) | 3,952 ( 1.04 ) | 287 ( 1.1 ) | 3,665 ( 1.03 ) | 11 ( 1.34 ) | 3,941 ( 1.04 ) |
| Prescribed neither methotrexate nor folic acid, n(%) | 363,821 ( 95.65 ) | 24,462 ( 93.97 ) | 339,359 ( 95.77 ) | 689 ( 84.02 ) | 363,132 ( 95.67 ) |
| Rheumatoid arthritis, n(%) | 999 ( 0.26 ) | 97 ( 0.37 ) | 902 ( 0.25 ) | 8 ( 0.98 ) | 991 ( 0.26 ) |
| Sickle cell disease, n(%) | 517 ( 0.14 ) | 51 ( 0.2 ) | 466 ( 0.13 ) | 2 ( 0.24 ) | 515 ( 0.14 ) |
| Prescribed anticonvulsant medication, n(%) | 1,642 ( 0.43 ) | 120 ( 0.46 ) | 1,522 ( 0.43 ) | 10 ( 1.22 ) | 1,632 ( 0.43 ) |
| Prescribed statins, n(%) | 156,064 ( 41.03 ) | 10,398 ( 39.94 ) | 145,666 ( 41.11 ) | 521 ( 63.54 ) | 155,543 ( 40.98 ) |
| Prescribed iron supplements, n(%) | 18,471 ( 4.86 ) | 1,661 ( 6.38 ) | 16,810 ( 4.74 ) | 106 ( 12.93 ) | 18,365 ( 4.84 ) |
| BMI, mean(sd) | 27.41 ( 4.76 ) | 28.12 ( 5.03 ) | 27.35 ( 4.74 ) | 30.21 ( 5.79 ) | 27.4 ( 4.76 ) |
| Townsend deprivation index, mean (sd) | -1.35 ( 3.04 ) | -0.9 ( 3.18 ) | -1.39 ( 3.03 ) | -0.28 ( 3.4 ) | -1.36 ( 3.04 ) |
| Never smoked, n(%) | 210,993 ( 55.47 ) | 13,990 ( 53.74 ) | 197,003 ( 55.6 ) | 321 ( 39.15 ) | 210,672 ( 55.5 ) |
| Current smoker, n(%) | 132,222 ( 34.76 ) | 9,262 ( 35.58 ) | 122,960 ( 34.7 ) | 384 ( 46.83 ) | 131,838 ( 34.73 ) |
| Former smoker, n(%) | 37,165 ( 9.77 ) | 2,781 ( 10.68 ) | 34,384 ( 9.7 ) | 115 ( 14.02 ) | 37,050 ( 9.76 ) |

Association with a diagnosis of COVID-19

Compared with people prescribed neither folic acid nor methotrexate, individuals prescribed folic acid had significant association with diagnosis of COVID-19 in Model 1 (OR 1.60 [1.50 ; 1.70]) (Table 2). In Model 2, which included a diagnosis of RA, sickle cell disease, and prescription of anticonvulsants or statins or iron supplements, this association was not attenuated (OR 1.51 [1.42 ; 1.61]). The prescription of methotrexate without folic acid was uncommon (only 174 people) and did not show an association with COVID-19 diagnosis in either Model. The prescription of methotrexate in combination with folic acid was associated with an increased risk for a diagnosis of COVID-19 in Model 1 (1.15 [ 1.02 ; 1.30 ]) but not in Model 2 (1.09 [0.96 ; 1.23 ]) (Table 2 and Table S2). The risk for COVID-19 diagnosis was associated with similar magnitudes with the prescription of folic acid in men and women in Model 2 (OR 1.50 [1.37 ; 1.64] and 1.52 [1.39 ; 1.65], respectively) (Table S3). The Model 2 sex-specific associations were not statistically significant and of similar magnitudes with methotrexate and with methotrexate combined with folic acid.

Table 2. COVID-19 diagnosis in people prescribed methotrexate and / or folic acid in the UKBB, compared to people not prescribed methotrexate or folic acid

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Neither Folic acid nor Methotrexate N= 363,821 | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only N= 12,433 | 1.58 [1.49 ; 1.68 ] | <0.001 | 1.60 [1.50 ; 1.70] | <0.001 | 1.51 [1.42 ; 1.61] | <0.001 |
| Methotrexate only N=174 | 0.94 [0.51 ; 1.72] | 0.83 | 0.89 [0.48 ; 1.65] | 0.72 | 0.86 [0.47 ; 1.6] | 0.64 |
| Methotrexate and Folic acid N= 3,952 | 1.09 [0.96 ; 1.23] | 0.18 | 1.15 [1.02 ; 1.30] | 0.021 | 1.09 [0.96 ; 1.23] | 0.18 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Association with mortality related to a COVID-19 diagnosis

In the general population, compared with people prescribed neither folic acid nor methotrexate, individuals prescribed folic acid had a significant association with mortality related to COVID-19 in Model

1 (OR 2.91 [2.38 ; 3.55]) (Table 3 and Table S4). In Model 2, which included a diagnosis of RA, sickle cell disease, prescription of anticonvulsants, statins and iron supplements, this association was maintained (OR 2.64 [2.15 ; 3.24]). Although there was a higher proportion of methotrexate prescriptions in the group that died with COVID-19, there were no deaths reported in individuals diagnosed with COVID-19 who were prescribed only methotrexate (N = 11). Moreover, the prescription of methotrexate in combination with folic acid was not associated with an increased odds for death after diagnosis of COVID-19 in Model 1 (Table 3) (1.26 [ 0.70 ; 2.30 ]) or Model 2 (1.07 [0.57 ; 1.98 ]). The risk for mortality after COVID-19 diagnosis was of similar magnitude with the prescription of folic acid in both men and women in Model 2 (OR 2.59 [2.00 ; 3.36] and 2.72 [1.93 ; 3.84], respectively) (Table S5). In both men and women co-prescription of methotrexate attenuated the association.

Table 3. The association of prescription of methotrexate and folic acid with COVID-19-related death in the UK Biobank*

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither Folic acid nor Methotrexate N= 363,821 | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only N= 12,433 | 5.14 [4.23 ; 6.24] | <0.001 | 2.91 [2.38 ; 3.55] | <0.001 | 2.64 [2.15 ; 3.24] | <0.001 |
| Methotrexate and folic acid N= 3,952 | 1.47 [0.81 ; 2.67] | 0.21 | 1.26 [0.70 ; 2.30] | 0.44 | 1.07 [0.57 ; 1.98] | 0.84 |

*There were no deaths in the group of participants taking only methotrexate without folate

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

To account for improvements in outcome of patients with COVID-19, resulting from changes in public health measures and emergence of different SARS-CoV-2 lineages over time [16] we tested for association with death in the COVID-19-positive cohort including also a quarterly (3-monthly) categorical time variable for diagnosis of COVID-19 using Model 2 (Table S6). This revealed a similar pattern of association with death - there was association with increased risk of death in patients prescribed folic acid only (OR 1.46 [1.16 ; 1.83]) but not in the group prescribed both folic acid and methotrexate (OR 0.96 [0.50 ; 1.83]).

**Discussion**

In this population-based analysis, we report 1.5-fold increased risk for COVID-19 diagnosis and 2.6-fold increased risk for COVID-19-related death among those who had been prescribed folic acid supplementation. The prescription of methotrexate was not associated with an increased risk of diagnosis of COVID-19 and we were not able to make an estimate for COVID-19-related death in the small sample of those prescribed methotrexate only. Notably, those prescribed methotrexate and folic acid did not have an increased risk for COVID-19 diagnosis or associated death, indicating that methotrexate might attenuate an increased risk for COVID-19 diagnosis and related-death conferred by folic acid.

In the context of SARS-Cov-2 infection it is established that hijacking of cellular metabolic pathways is important for viral replication.[17] Zhang et al described that SARS-CoV-2 remodels host folate and one-carbon metabolism at the post-transcriptional level to support de novo purine synthesis, bypassing viral shutoff of host translation. [8] This suggests that viral replication could be sensitive to folate inhibitors, such as methotrexate. Intracellular glucose and folate are depleted in SARS-CoV-2-infected cells, and viral replication is exquisitely sensitive *in vitro* to inhibitors of folate and one carbon metabolism, notably methotrexate.[8] Stegmann et al, based on cell culture experiments, reported that methotrexate alone or in combination with remdesivir limits the replication of SARS-CoV-2.[18] With the caveat that our study is observational epidemiology and causality cannot be inferred, our study does support the possibility that external folate supply facilitates the production of large amounts of virus, contributing to clinical infection and mortality. Our study also supports the notion that SARS-CoV-2 replication is enhanced by folate supply based on our finding that co-prescription of an antifolate (methotrexate) can ameliorate the possibly adverse effect of supplementation with folic acid on COVID-19 outcomes.

There is also evidence that inadequate folate status may be harmful in the context of host resistance to infection with SARS-CoV-2. In addition to the well-recognized complication of anemia, folate deficiency has other detrimental health effects, including suppression of immune function.[19] Additional support for the concept that adequate folate status is important in COVID-19 outcomes is provided by the observation that folate deficiency was associated with poorer outcomes in a cohort of COVID-19 patients.[20] It is important to note that it is possible that in the study by Itelman et al,[20] if increased folate levels were causal of COVID-19 diagnosis and poor outcomes, that the association with lower folate levels could have been caused by selection (collider) bias.[21] Vitamin B12 deficiency has also been proposed as a factor related to poor COVID-19 outcomes, presumed to be through the induction of functional folate deficiency.[22] A drug-protein structure interaction analysis raises the possibility that folate blocks the 3CL hydrolase enzyme, which may affect viral entry and replication.[23] It is therefore possible that both inadequate and excessive amounts of folate may be detrimental to host resistance to SARS-CoV-2 infection and that there may be an optimal range of physiological folate status related to host-resistance to COVID-19 infection and severity.

Data from the COVID-19 Global Rheumatology Alliance describes that a number of immunomodulatory drugs used in rheumatology are associated with an increased risk of infection and death compared with methotrexate. [7] Being on no DMARD therapy was associated with an increased risk of death with COVID-19 (OR 2.11 [1.48 ; 3.01]), which could be interpreted as either a protective effect of methotrexate or an increased risk for death associated with poor rheumatic disease control. The authors of the study additionally noted that people not on DMARD therapy had increased use of glucocorticoids meaning that confounding by indication cannot be ruled out as an explanation.[24] Methotrexate was also associated with lower odds for death when compared with sulfasalazine, other immunosuppressants and rituximab. In no case was methotrexate associated with an increased risk for death. The COVID-19 Global Rheumatology Alliance study did not explore the effect of folic acid supplementation in the

setting of methotrexate, although it is it is highly likely that almost all patients on methotrexate also were receiving folic acid supplementation. Considering the widespread use of folic acid supplements and proposals to abandon entirely tolerable upper intake levels for folic acid [25] it would be prudent to monitor the effect of increased folic acid intake at a population level on COVID-19 morbidity and mortality, particularly at the upper end of folic acid intake.

Several limitations of our analysis are important to note. One, given the small size of the methotrexate-only group and that there were no deaths related to COVID-19 in this group we could not test a beneficial effect on mortality of methotrexate in isolation. It is uncommon to find patients with methotrexate prescribed without supplemental folic acid as this is the standard of care. Second, over the time period of this study (March 2020 – November 2021), COVID-19 outcomes (i.e. death) will have been influenced by the development of clinical treatments including antiviral drugs and monoclonal antibodies, changes to public health measures and the appearance of new COVID-19 strains [16] . We were unable to account for these factors in the population-based analysis however we attempted to account for this in the analysis within the COVID-19-positive group by including a time variable (Table S4). Third, findings are not necessarily generalizable outside of the middle-aged (>45 years of age) white European cohort that dominates the UK Biobank. Fourth, the full extent of SARS-CoV-2 infection is not known in the UK population due to incomplete testing rates early in the pandemic. Fifth, prescription data were single script from General Practitioners only, and it was not possible to ascertain compliance or whether participants were taking the prescribed medication during the COVID-19 pandemic or continuously during the study period although we attempted to account for this by restricting our use of prescription information to the years 2019 and 2020 only. Sixth, although we included rheumatoid arthritis in Model 2, we were unable to account for any potential effect of disease activity in RA in people prescribed folic acid. Rheumatoid arthritis disease activity negatively impacts death from COVID-19 outcomes.[7] Seventh, while it is a strength of our study that mandatory fortification of the UK diet with folic acid had not been

introduced during the period of our study and thus did not confound our analysis, we were unable to account for the lower-dose over the counter folic acid supplementation available in the UK (400 micrograms being the most common formulation for over the counter tablets) because there were no self-report information in the UK Biobank dataset on the use of folic acid supplementation. Finally, residual confounding conferred by the underlying indications for folate prescriptions (besides the ones addressed in our analysis) is a possibility

In conclusion, and despite the limitations of our study enumerated above, our data support the hypothesis that increased folate resulting from folic acid prescription could contribute to a higher probability of contracting clinically-detectable infection with SARS-CoV2 and to an increase in the risk of death following the infection. The study population was drawn from the >45 year old segment of the UK population and is predominantly of white European ethnicity, therefore our findings have reduced generalizability to younger people, to other ethnic groups and to other countries. Nevertheless, our findings justify future studies on the influence of folic acid supplementation on COVID-19 outcomes, particularly in pregnant women and people on anticonvulsants requiring supplementary folic acid.

As a final comment, we point out that attention is currently being directed toward establishing whether excessive intake of folate, particularly in the form of folic acid, may have undesirable and potentially deleterious effects.[26] The possibility that susceptibility to COVID-19 infection and its serious and even fatal complications may be affected by folic acid intake and folate status should be thoroughly investigated.

**Acknowledgements**

This research was conducted using the UK Biobank Resource under Application Number 12611. We sincerely thank all participants.

**Declarations of interest**

PCR reports personal fees from Abbvie, Atom Biosciences, Eli Lilly, Gilead, Janssen, Novartis, UCB, Roche, Pfizer; meeting attendance support from BMS, Pfizer and UCB Pharma and grant funding from Janssen, Novartis, Pfizer and UCB Pharma, all outside the submitted work. AG reports personal fees from SOBI, Selecta and honoraria from UptoDate, Inc. outside the submitted work. All other authors have no declarations of interest.

**Author contributions**

RKT, RG, SLM, PCR, TRM and ALG substantially contributed to study conception and design, to acquisition and analysis of data and interpretation of results. RKT, RG, SLM, PCR, TRM and ALG contributed to drafting the article and critical revision and RKT, RG, SLM, PCR, TRM and ALG approved the final version. RKT and TRM directly accessed and verified the underlying data reported in the manuscript.

**Figure legends**

**Figure 1 Flow schematic of study design**

**Figure 2 Data sources of COVID-19-diagnosed individuals**

Of the 26,033 COVID-19-diagnosed individuals, 25,300 were identified from positive SARS-Cov2 test results (21,705 unique to this group), 4,170 identified from hospital records (650 unique to this group), and 820 identified from death records (51 unique to this group). 217 diagnosed after 13th October 2021 (30 days before the last recorded death) were removed from the case only analysis in Table S4 given the unknown outcome.

**Funding Statement**

There was no funding to report for this research study

REFERENCES


1. Department of Health and Social Care UG. [Available from: https://www.gov.uk/government/news/folic-acid-added-to-flour-to-prevent-spinal-conditions-in-babies accessed 1/11 2022.
2. Dixit R, Nettem S, Madan SS, et al. Folate supplementation in people with sickle cell disease. *Cochrane Database Syst Rev* 2018;3:CD011130. doi: 10.1002/14651858.CD011130.pub3 [published Online First: 2018/03/17]
3. Linnebank M, Moskau S, Semmler A, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol* 2011;69(2):352-9. doi: 10.1002/ana.22229 [published Online First: 2011/01/20]
4. Fraenkel L, Bathon JM, England BR, et al. 2021 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol* 2021;73(7):1108-23. doi: 10.1002/art.41752 [published Online First: 2021/06/09]
5. Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;121(11):833-41. doi: 10.7326/0003-4819-121-11-199412010-00002 [published Online First: 1994/12/01]
6. Morgan SL, Baggott JE, Vaughn WH, et al. The effect of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 1990;33(1):9-18. doi: 10.1002/art.1780330102 [published Online First: 1990/01/01]
7. Strangfeld A, Schäfer M, Gianfrancesco MA, et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Annals of the Rheumatic Diseases* 2021;80(7):930-42. doi: 10.1136/annrheumdis-2020-219498
8. Zhang Y, Guo R, Kim SH, et al. SARS-CoV-2 hijacks folate and one-carbon metabolism for viral replication. *Nature Communications* 2021;12(1):1676. doi: 10.1038/s41467-021-21903-z
[Dataset] 9. [Available from: https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access accessed June 22 2022.
10. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12(3):e1001779. doi: 10.1371/journal.pmed.1001779 [published Online First: 2015/04/01]
11. Moore JL. The significance of folic acid for epilepsy patients. *Epilepsy Behav* 2005;7(2):172-81. doi: 10.1016/j.yebeh.2005.05.020 [published Online First: 2005/08/02]
12. Girelli D, Marchi G, Busti F, et al. Iron metabolism in infections: Focus on COVID-19. *Semin Hematol* 2021;58(3):182-87. doi: 10.1053/j.seminhematol.2021.07.001 [published Online First: 2021/08/15]
13. Gonciarz RL, Renslo AR. Emerging role of ferrous iron in bacterial growth and host-pathogen interaction: New tools for chemical (micro)biology and antibacterial therapy. *Curr Opin Chem Biol* 2021;61:170-78. doi: 10.1016/j.cbpa.2021.01.015 [published Online First: 2021/03/15]
14. Diaz-Arocutipa C, Melgar-Talavera B, Alvarado-Yarasca A, et al. Statins reduce mortality in patients with COVID-19: an updated meta-analysis of 147 824 patients. *Int J Infect Dis* 2021;110:374-81. doi: 10.1016/j.ijid.2021.08.004 [published Online First: 2021/08/11]
15. Kollias A, Kyriakoulis KG, Kyriakoulis IG, et al. Statin use and mortality in COVID-19 patients: Updated systematic review and meta-analysis. *Atherosclerosis* 2021;330:114-21. doi: 10.1016/j.atherosclerosis.2021.06.911 [published Online First: 2021/07/11]

16. Davies NG, Jarvis CI, Group CC-W, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature* 2021;593(7858):270-74. doi: 10.1038/s41586-021-03426-1 [published Online First: 2021/03/17]
17. Thaker SK, Ch'ng J, Christofk HR. Viral hijacking of cellular metabolism. *BMC Biology* 2019;17(1):59. doi: 10.1186/s12915-019-0678-9
18. Stegmann KM, Dickmanns A, Gerber S, et al. The folate antagonist methotrexate diminishes replication of the coronavirus SARS-CoV-2 and enhances the antiviral efficacy of remdesivir in cell culture models. *Virus Res* 2021;302:198469. doi: 10.1016/j.virusres.2021.198469 [published Online First: 2021/06/07]
19. Green R, Miller JW. Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations of dysfunctional folate status. *Semin Hematol* 1999;36(1):47-64. [published Online First: 1999/02/04]
20. Itelman E, Wasserstrum Y, Segev A, et al. Clinical Characterization of 162 COVID-19 patients in Israel: Preliminary Report from a Large Tertiary Center. *Isr Med Assoc J* 2020;22(5):271-74. [published Online First: 2020/05/08]
21. Choi HK, Nguyen US, Niu J, et al. Selection bias in rheumatic disease research. *Nat Rev Rheumatol* 2014;10(7):403-12. doi: 10.1038/nrrheum.2014.36 [published Online First: 2014/04/02]
22. Wee AKH. COVID-19's toll on the elderly and those with diabetes mellitus - Is vitamin B12 deficiency an accomplice? *Med Hypotheses* 2021;146:110374. doi: 10.1016/j.mehy.2020.110374 [published Online First: 2020/12/02]
23. Serseg T, Benarous K, Yousfi M. Hispidin and Lepidine E: Two Natural Compounds and Folic Acid as Potential Inhibitors of 2019-novel Coronavirus Main Protease (2019- nCoVM(pro)), Molecular Docking and SAR Study. *Curr Comput Aided Drug Des* 2021;17(3):469-79. doi: 10.2174/1573409916666200422075440 [published Online First: 2020/04/24]
24. Schafer M, Strangfeld A, Hyrich KL, et al. Response to: 'Correspondence on 'Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician reported registry'' by Mulhearn et al. *Ann Rheum Dis* 2021 doi: 10.1136/annrheumdis-2021-220134 [published Online First: 2021/03/03]
25. Wald NJ, Morris JK, Blakemore C. Public health failure in the prevention of neural tube defects: time to abandon the tolerable upper intake level of folate. *Public Health Rev* 2018;39:2. doi: 10.1186/s40985-018-0079-6 [published Online First: 2018/02/17]
26. Maruvada P, Stover PJ, Mason JB, et al. Knowledge gaps in understanding the metabolic and clinical effects of excess folates/folic acid: a summary, and perspectives, from an NIH workshop. *Am J Clin Nutr* 2020;112(5):1390-403. doi: 10.1093/ajcn/nqaa259 [published Online First: 2020/10/07]

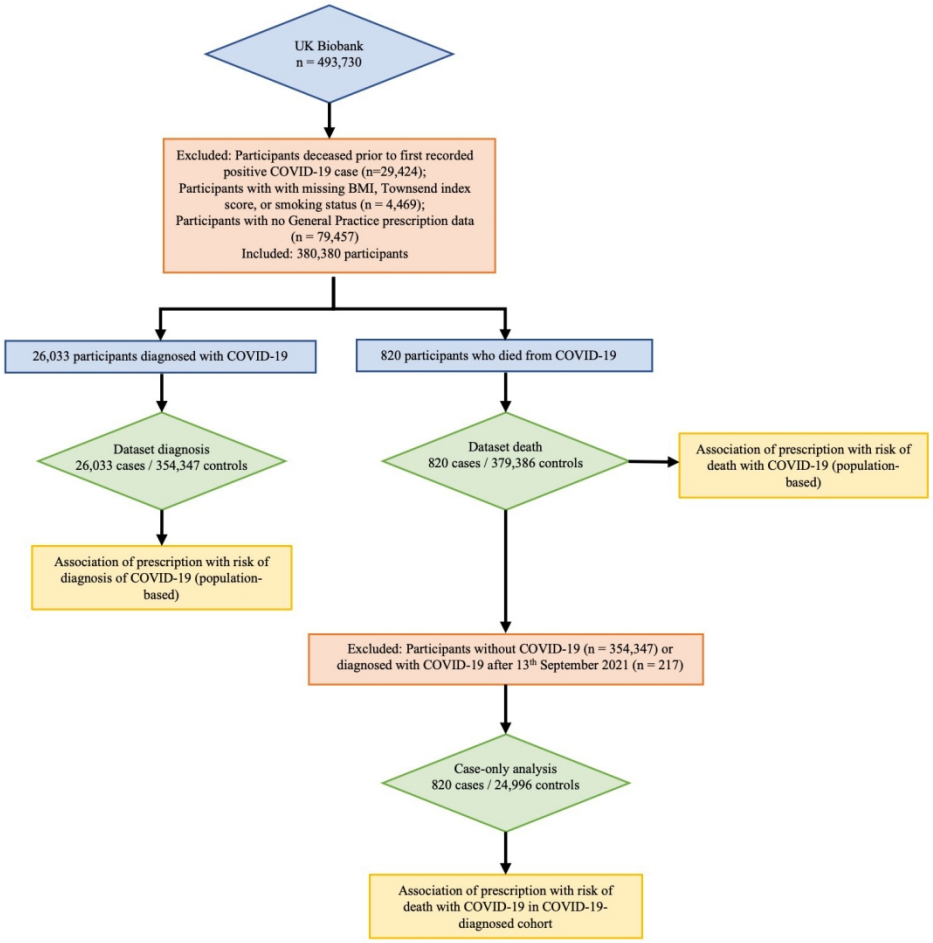


Figure 1 Flow schematic of study design

181x190mm (225 x 225 DPI)

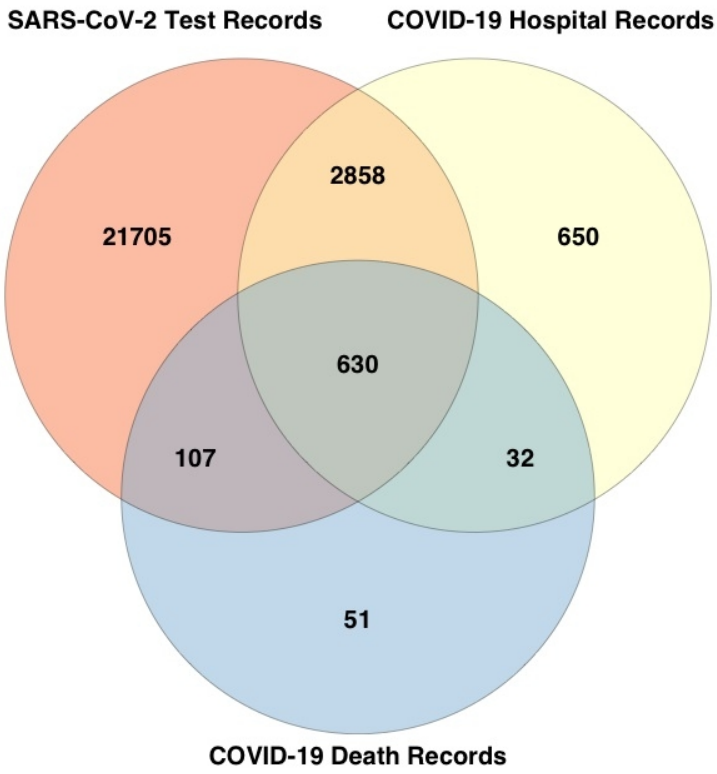


Figure 2 Data sources of COVID-19-diagnosed individuals. Of the 26,033 COVID-19-diagnosed individuals, 25,300 were identified from positive SARS-Cov2 test results (21,705 unique to this group), 4,170 identified from hospital records (650 unique to this group), and 820 identified from death records (51 unique to this group). 217 diagnosed after 13th October 2021 (30 days before the last recorded death) were removed from the case only analysis in Table S4 given the unknown outcome.

211x211mm (96 x 96 DPI)

Table S1. Medications co-prescribed with folic acid on >5% of occasions

| Medication/Supplement | Number of prescriptions* | Percentage of folic acid prescriptions |
|---|---|---|
| Folic acid | 177,918 | 100.00 |
| Methotrexate | 45,697 | 25.68 |
| Atorvastatin | 41,296 | 23.21 |
| Lansoprazole | 35,611 | 20.02 |
| Omeprazole | 32,909 | 18.50 |
| Bisoprolol | 26,445 | 14.86 |
| Levothyroxine | 25,482 | 14.32 |
| Ramipril | 21,990 | 12.36 |
| Aspirin | 21,696 | 12.19 |
| Paracetamol | 20,830 | 11.71 |
| Amlodipine | 18,354 | 10.32 |
| Simvastatin | 16,878 | 9.49 |
| Amitriptyline | 13,573 | 7.63 |
| Furosemide | 12,983 | 7.30 |
| Metformin | 11,460 | 6.44 |
| Hydroxychloroquine | 11,038 | 6.20 |
| Clopidogrel | 10,918 | 6.14 |
| Prednisolone | 10,475 | 5.89 |
| Alendronic acid | 10,198 | 5.73 |

*General practice prescription data from 1st of January 2019 through to 27th September 2021

Table S2. COVID-19 diagnosis in people prescribed methotrexate and / or folic acid in the UKBB, compared to people not prescribed methotrexate or folic acid

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Prescribed folic acid only[1] | 1.58 [1.49 ; 1.68 ] | <0.001 | 1.60 [ 1.5 ; 1.7 ] | <0.001 | 1.51 [ 1.42 ; 1.61 ] | <0.001 |
| Prescribed methotrexate only[1] | 0.94 [0.51 ; 1.72] | 0.83 | 0.89 [ 0.48 ; 1.65 ] | 0.72 | 0.86 [ 0.47 ; 1.6 ] | 0.64 |
| Prescribed methotrexate and folic acid[1] | 1.09 [0.96 ; 1.23] | 0.18 | 1.15 [ 1.02 ; 1.3 ] | 0.021 | 1.09 [ 0.96 ; 1.23 ] | 0.18 |
| 60-70 years of age[2] | | | 0.60 [ 0.58 ; 0.62 ] | <0.001 | 0.59 [ 0.57 ; 0.61 ] | <0.001 |
| 70-75 years of age[2] | | | 0.41 [ 0.4 ; 0.43 ] | <0.001 | 0.40 [ 0.38 ; 0.41 ] | <0.001 |
| >75 years of age[2] | | | 0.40 [ 0.38 ; 0.41 ] | <0.001 | 0.38 [ 0.37 ; 0.4 ] | <0.001 |
| Male sex | | | 1.09 [ 1.07 ; 1.12 ] | <0.001 | 1.09 [ 1.06 ; 1.12 ] | <0.001 |
| Former smoker[3] | | | 0.87 [ 0.84 ; 0.92 ] | <0.001 | 0.87 [ 0.83 ; 0.91 ] | <0.001 |
| Never smoked[3] | | | 0.88 [ 0.86 ; 0.91 ] | <0.001 | 0.89 [ 0.86 ; 0.91 ] | <0.001 |
| BMI | | | 1.03 [ 1.03 ; 1.03 ] | <0.001 | 1.03 [ 1.03 ; 1.03 ] | <0.001 |
| Asian British[4] | | | 1.38 [ 1.29 ; 1.48 ] | <0.001 | 1.33 [ 1.25 ; 1.43 ] | <0.001 |
| Black British[4] | | | 1.08 [ 1 ; 1.17 ] | 0.057 | 1.08 [ 1 ; 1.18 ] | 0.058 |
| Other ethnicity[4] | | | 1.05 [ 0.95 ; 1.15 ] | 0.35 | 1.04 [ 0.95 ; 1.15 ] | 0.40 |
| Townsend deprivation index | | | 1.03 [ 1.02 ; 1.03 ] | <0.001 | 1.03 [ 1.02 ; 1.03 ] | <0.001 |
| Rheumatoid arthritis | | | | | 1.4 [ 1.12 ; 1.74 ] | 0.003 |
| Sickle cell disease | | | | | 1.00 [ 0.74 ; 1.35 ] | 1.00 |
| Prescribed anticonvulsant medication | | | | | 1.07 [ 0.89 ; 1.29 ] | 0.48 |
| Prescribed statins | | | | | 1.08 [ 1.05 ; 1.12 ] | <0.001 |
| Prescribed iron supplements | | | | | 1.29 [ 1.22 ; 1.36 ] | <0.001 |

1 Compared to Prescribed neither methotrexate nor folic acid; 2 Compared to <60 years of age; Compared to Current smoker; 4 Compared to White British; Model 1 and Model 2 contain all variables with odds ratios present (and comparative groups) as respectively listed below the titles.

Table S3. Sex-specific associations between prescription of methotrexate, folic acid, and the combination of both with the risk of COVID-19

| | COVID-19 diagnosis - Male | | | | | | COVID-19 diagnosis -Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | | *Model 1* | | *Model 2* | | *Unadjusted* | | *Model 1* | | *Model 2* | |
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | OR, [95% CI] | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 1.52 [ 1.39 ; 1.66 ] | <0.001 | 1.57 [ 1.44 ; 1.72 ] | <0.001 | 1.50 [ 1.37 ; 1.64 ] | <0.001 | 1.64 [ 1.51 ; 1.77 ] | <0.001 | 1.61 [ 1.48 ; 1.75 ] | <0.001 | 1.52 [ 1.39 ; 1.65 ] | <0.001 |
| Methotrexate only | 0.96 [ 0.39 ; 2.38 ] | 0.93 | 0.93 [ 0.37 ; 2.32 ] | 0.88 | 0.92 [ 0.37 ; 2.30 ] | 0.86 | 0.92 [ 0.4 ; 2.11 ] | 0.85 | 0.87 [ 0.38 ; 1.99 ] | 0.74 | 0.83 [ 0.36 ; 1.90 ] | 0.65 |
| Methotrexate and folic acid | 1.08 [ 0.89 ; 1.31 ] | 0.45 | 1.15 [ 0.94 ; 1.40 ] | 0.17 | 1.11 [ 0.91 ; 1.36 ] | 0.29 | 1.11 [ 0.95 ; 1.29 ] | 0.19 | 1.16 [ 0.99 ; 1.36 ] | 0.060 | 1.07 [ 0.91 ; 1.26 ] | 0.38 |

diagnosis

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status
Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Table S4. The association of prescription of methotrexate and folic acid with COVID-19-related death in the UK Biobank.

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Prescribed folic acid only[1] | 1.47 [0.81 ; 0.67] | 0.21 | 2.91 [ 2.38 ; 3.55 ] | <0.001 | 2.64 [ 2.15 ; 3.24 ] | <0.001 |
| Prescribed methotrexate only[1] | N/A | N/A | N/A | N/A | N/A | N/A |
| Prescribed methotrexate and folic acid[1] | 5.14 [4.23 ; 6.24] | <0.001 | 1.26 [ 0.7 ; 2.3 ] | 0.44 | 1.07 [ 0.57 ; 1.98 ] | 0.84 |
| 60-70 years of age[2] | | | 3.05 [ 2 ; 4.65 ] | <0.001 | 2.98 [ 1.95 ; 4.56 ] | <0.001 |
| 70-75 years of age[2] | | | 5.70 [ 3.76 ; 8.64 ] | <0.001 | 5.46 [ 3.59 ; 8.31 ] | <0.001 |
| >75 years of age[2] | | | 14.7 [ 9.86 ; 21.91 ] | <0.001 | 13.87 [ 9.26 ; 20.78 ] | <0.001 |
| Male sex | | | 2.13 [ 1.84 ; 2.46 ] | <0.001 | 2.13 [ 1.84 ; 2.47 ] | <0.001 |
| Former smoker[3] | | | 1.37 [ 1.11 ; 1.7 ] | 0.004 | 1.37 [ 1.11 ; 1.7 ] | 0.004 |
| Never smoked[3] | | | 0.76 [ 0.65 ; 0.89 ] | <0.001 | 0.77 [ 0.66 ; 0.89 ] | 0.001 |
| BMI | | | 1.09 [ 1.08 ; 1.11 ] | <0.001 | 1.09 [ 1.08 ; 1.11 ] | <0.001 |
| Asian British[4] | | | 2.48 [ 1.76 ; 3.49 ] | <0.001 | 2.33 [ 1.65 ; 3.29 ] | <0.001 |
| Black British[4] | | | 1.74 [ 1.15 ; 2.63 ] | <0.001 | 1.72 [ 1.13 ; 2.63 ] | 0.012 |
| Other ethnicity[4] | | | 1.44 [ 0.86 ; 2.42 ] | 0.16 | 1.44 [ 0.86 ; 2.41 ] | 0.17 |
| Townsend deprivation index | | | 1.09 [ 1.07 ; 1.11 ] | <0.001 | 1.08 [ 1.06 ; 1.11 ] | <0.001 |
| Rheumatoid arthritis | | | | | 2.48 [ 1.19 ; 5.17 ] | 0.015 |
| Sickle cell disease | | | | | 1.22 [ 0.29 ; 5.15 ] | 0.78 |
| Prescribed anticonvulsant medication | | | | | 2.22 [ 1.18 ; 4.17 ] | 0.014 |
| Prescribed statins | | | | | 1.08 [ 0.93 ; 1.25 ] | 0.33 |
| Prescribed iron supplements | | | | | 1.44 [ 1.16 ; 1.79 ] | 0.001 |

1 Compared to Prescribed neither methotrexate nor folic acid; 2 Compared to <60 years of age; Compared to Current smoker; 4 Compared to White British; N/A = not applicable due to no deaths in the methotrexate only group. Model 1 and Model 2 contain all variables with odds ratios present (and comparative groups) as respectively listed below the titles.

Table S5. Sex-specific associations between prescription of methotrexate, folic acid, and the combination of both with the risk of COVID-19 related mortality.

| | COVID-19 related death – Male | | | | | | COVID-19 related death - Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | | *Model 1* | | *Model 2* | | *Unadjusted* | | *Model 1* | | *Model 2* | |
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | OR, [95% CI] | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 4.73 [3.71;6.04] | <0.001 | 2.83 [2.20;3.64 ] | <0.001 | 2.59 [2.00;3.36] | <0.001 | 5.67 [4.10;7.82 ] | <0.001 | 3.07 [2.20; 4.28] | <0.001 | 2.72 [1.93;3.84] | <0.001 |
| Methotrexate and folic acid | 2.00 [0.99;4.03] | 0.053 | 1.62 [0.80;3.26] | 0.18 | 1.54 [0.75;3.15] | 0.24 | 1.01 [0.32;3.16] | 0.99 | 0.80 [0.25;2.50] | 0.70 | 0.56 [0.17;1.84] | 0.34 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status
Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Table S6. Risk of death related to COVID-19 in the COVID-19-positive group

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 3.58 [ 2.93 ; 4.39 ] | <0.001 | 1.82 [ 1.47 ; 2.26 ] | <0.001 | 1.46 [ 1.16 ; 1.83 ] | 0.0011 |
| Methotrexate and folic acid | 1.37 [ 0.75 ; 2.52 ] | 0.31 | 1.03 [ 0.55 ; 1.91 ] | 0.94 | 0.96 [ 0.50 ; 1.83 ] | 0.90 |

Model 2 additionally adjusted by quarterly categorical time variable.

A total of 25,816 COVID-19-positive cases were analyzed. 217 COVID-19-positive cases who were diagnosed after 13th September 2021 (28 days before the last recorded death) were removed from the cohort given the unknown outcome in these individuals

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 3 | 'A population-based cohort' in third line of the abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 3 | Throughout abstract |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 6-7 | All text in Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 7 | Last sentence of Introduction. |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | 3,7 | Refer items 1a, 1b and 3 above. |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-9 | Throughout the methods |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 8-9 (case-control) | This information is in the Methods sections 'COVID-19 definitions' and 'Prescription data' |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | | Matching was not done in this study. |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 8 | This information is in the Methods section 'Ethnicity, age and comorbidity data'. |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 8-9 | This information is in the Methods sections 'Gout and COVID-19 definitions and case- |

| | | | | |
|---|---|---|---|---|
| | | | | control datasets' and 'Ethnicity, age and comorbidity data'. |
| Bias | 9 | Describe any efforts to address potential sources of bias | Table S4 | This analysis including a categorical time variable was done to account for improvements in treatment over time |
| Study size | 10 | Explain how the study size was arrived at | | The study size comprised all available data from the UK Biobank. |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | | Quantitative variables were BMI, age and Townsend deprivation index. These were all included as potential confounding variables. Age groupings described in the final paragraph on page 6. |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 9 | Refer to Methods section 'Statistical analysis'. |
| | | (*b*) Describe any methods used to examine subgroups and interactions | | The COVID-19 case-only analysis was one subgroup (Table S4). Additional subgroups were men and women. |
| | | (*c*) Explain how missing data were addressed | 8 | Paragraph 1: "there were 464,306 participants, of whom 4,469 were removed owing to not having a BMI measure or Townsend index score or smoking status and a further 112,466 were removed owing to lack of prescription data." |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | | Not applicable |
| | | (*e*) Describe any sensitivity analyses | | No sensitivity analyses were done. |
| **Results** | | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | | Refer Figure 1. |
| | | (b) Give reasons for non-participation at each stage | 8 | Refer paragraph 1. |
| | | (c) Consider use of a flow diagram | | Figure 1 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 18-19 | Table 1 |

| | | | | |
|---|---|---|---|---|
| | | (b) Indicate number of participants with missing data for each variable of interest | | Not applicable. Participants with missing data were excluded. |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | | Not applicable |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | | Not applicable. |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | 18-19 | Table 1 |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 9-10,20-21 | Unadjusted estimates are provided (Tables 2,3). The reason for inclusion of potential confounders in Model 2 is explained in last paragraph pg 9 and first paragraph pg 10. |
| | | (*b*) Report category boundaries when continuous variables were categorized | | This was done for age: "Age was calculated for 2020 from year of birth. Age groups used in the analysis were <60 years (n= 57,618), 60-69 years (n= 107,140), 70-74 years (n= 85,926) and >74 years (n= 96,687)." |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | | Not applicable |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 23-24,26 | Analyses stratified by sex and vaccination status reported in Tables S2, S3, S5. |
| **Discussion** | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 | First paragraph: "In this population-based analysis, we report 1.7-fold increased risk for COVID-19 diagnosis and 3.2-fold increased risk for COVID-19-related death among those having been prescribed folic acid supplementation. The prescription of methotrexate was not associated with an increased risk of diagnosis of COVID-19" |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15-16 | Eight specific limitations are discussed |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-14 | First three paragraphs of Discussion |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 | Paragraph 2: "findings are not necessarily generalizable outside of the middle-aged (>45 years of age) white European cohort that dominates the UK Biobank." |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 7 | Not applicable |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

# Folic acid and methotrexate use and their association with COVID-19 diagnosis and mortality: a case-control analysis from the UK Biobank



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2022-062945.R2 |
| Article Type: | Original research |
| Date Submitted by the Author: | 22-Jul-2022 |
| Complete List of Authors: | Topless, Ruth; University of Otago<br>Green, Ralph; University of California Davis at Sacramento, Departments of Pathology and Medicine<br>Morgan, Sarah; The University of Alabama at Birmingham<br>Robinson, Philip ; University of Queensland Faculty of Medicine and Biomedical Sciences<br>Merriman, Tony; University of Otago, School of Medical Sciences<br>Gaffo, Angelo; The University of Alabama at Birmingham; Birmingham VA Medical Center |
| Primary Subject Heading: | Epidemiology |
| Secondary Subject Heading: | Rheumatology, Infectious diseases |
| Keywords: | COVID-19, Epidemiology < INFECTIOUS DISEASES, Rheumatology < INTERNAL MEDICINE |
| | |

*I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.*

*The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.*

*Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.*

**Folic acid and methotrexate use and their association with COVID-19 diagnosis and mortality: a case-control analysis from the UK Biobank**

Ruth K Topless BSc [1], Ralph Green MD PhD FRCPath[2] , Sarah L. Morgan MD RD[3], Philip C Robinson MBChB PhD [4], Tony R Merriman PhD[1,3] , Angelo L. Gaffo MD MsPH FACP [3,5]

[1]Department of Biochemistry, University of Otago, Dunedin, New Zealand.

[2]Departments of Pathology and Medicine, University of California Davis at Sacramento, Sacramento, California, USA.

[3]Division of Clinical Immunology and Rheumatology, The University of Alabama at Birmingham, Birmingham, Alabama, USA.

[4]School of Clinical Medicine, University of Queensland, Brisbane, Australia

[5]Birmingham VA Medical Center. Birmingham, Alabama, USA

**Correspondence to:**

Angelo Gaffo

SHEL 306

1720 2nd Ave South

Birmingham, AL 35294.

USA

Phone: +1-205-9348909

Fax: +1-205-9966788

agaffo@uabmc.edu

Ralph Green

4400 V St.

Sacramento, CA 95817

Phone: +1-916-7348078

Fax: +1-916 – 7340299

rgreen@ucdavis.edu

**Abstract**

**Objective**: To determine if methotrexate or folic acid prescription were associated with differential risk for COVID-19 diagnosis or mortality.

**Design**: Case-control analysis.

**Setting**: The population-based UK Biobank (UKBB) cohort.

**Participants**: Data from 380,380 UKBB participants with general practice prescription data for 2019 to 2021. Updated medical information was retrieved on the 13th December 2021.

**Primary and secondary outcome measures**: The outcomes of COVID-19 diagnosis and COVID-19 related mortality were analyzed by multivariable logistic regression. Exposures evaluated were prescription of folic acid and/or methotrexate. Criteria for COVID-19 diagnosis were 1) a positive SARS-CoV-2 test or 2) ICD-10 code for confirmed COVID-19 (U07.1) or probable COVID-19 (U07.2) in hospital records, or death records. By these criteria 26,003 individuals were identified with COVID-19 of whom 820 were known to have died from COVID-19. Logistic regression statistical models were adjusted for age sex, ethnicity, Townsend deprivation index, BMI, smoking status, presence of rheumatoid arthritis, sickle cell disease, use of anticonvulsants, statins and iron supplements.

**Results**: Compared with people prescribed neither folic acid nor methotrexate, people prescribed folic acid supplementation had increased risk of diagnosis of COVID-19 (OR 1.51 [1.42 ; 1.61]). The prescription of methotrexate with or without folic acid was not associated with COVID-19 diagnosis (P≥0.18). People prescribed folic acid supplementation had positive association with death after a diagnosis of COVID-19 (OR 2.64 [2.15 ; 3.24]) in a fully adjusted model. The prescription of methotrexate in combination with folic acid was not associated with an increased risk for COVID-19 related death (1.07 [0.57 ; 1.98]).

**Conclusions**: We report an association of increased risk for COVID-19 diagnosis and COVID-19-related death in people prescribed folic acid supplementation. Our results also suggest that methotrexate might attenuate these associations.

**Strengths and limitations of this study**

- A strength of the study is the use of a large population-based cohort with linked data.
- Another strength is that the cohort was drawn from a population where food was not fortified with folic acid.
- A limitation of the use of prescription data is that it was not possible to assess compliance or to account for over-the-counter supplementation of folic acid.
- The findings cannot be generalized outside of the middle-aged (>45 years of age) white European demographic that dominates the UK Biobank cohort.
- The observational nature of the data means that causality cannot be inferred from our findings.

**Introduction**

Folate, a B-vitamin, carries out critical roles in the transfer of one-carbon units in intermediary metabolism. Folates exist in various forms depending on the one-carbon substituent attached to the parent molecule and are involved in numerous reactions, including the synthesis of methionine from homocysteine and are also utilized in purine and pyrimidine metabolism for DNA and RNA synthesis. The oxidized form, folic acid, is presently added to fortified foods in the USA and over 80 other countries. Recently, a decision has been taken in the UK to introduce fortification to prevent neural tube defect pregnancies. Folic acid also is used in dietary supplements to prevent or treat folate deficiency.[1] Additionally, folic acid supplementation of up to 5mg daily is often advised during pregnancy and in women of childbearing age and for other medical conditions (sickle cell anemia) [2] and during treatment with certain anticonvulsants [3].

Methotrexate, a structural analogue of folate has potent antifolate activity and is in widespread use as an antineoplastic agent and as a first-line disease-modifying antirheumatic drug (DMARD) treatment for rheumatoid arthritis (RA).[4] Folic acid (at doses commonly ranging from 1-2 mg daily) or folinic acid supplementation is often included to lower the toxicity of low-dose methotrexate therapy. [5 6]

The COVID-19 Global Rheumatology Alliance physician-reported registry has evaluated factors related to death from COVID-19 in individuals with rheumatic diseases.[7] Compared with those receiving methotrexate monotherapy, use of rituximab (OR 4.0 [95% CI 2.3 ; 7.0]), sulfasalazine (3.6 [1.7 ; 7.8]), azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and tacrolimus (2.2 [1.4 – 3.4]) or no DMARD (2.1 [1.5 – 3.0]) all had higher risks of death from COVID-19.

In order to generate purines SARS-CoV-2 post-transcriptionally remodels host folate metabolism. In an *in vitro* system using African green monkey kidney cells infected with SARS-CoV-2 intracellular glucose and folate were depleted, and this perturbation was sensitive to folate inhibitors such as methotrexate.

[8] It is therefore plausible that methotrexate therapy for RA could have a beneficial effect on COVID-19 outcomes given its antifolate activity. However, since folic acid is routinely included with methotrexate to prevent methotrexate-related toxicity, such putative beneficial effect of methotrexate on viral proliferation and hence on COVID-19 outcomes may be negated by folic acid supplementation.

The aim of this study was to determine whether the use of methotrexate and folic acid prescription, together or individually, were associated with a lowered or increased risk, respectively, for COVID-19 diagnosis or mortality in a large population based-cohort.

## Methods

### Data source

This research was conducted using the UK Biobank Resource (approval number 12611).[9] The UK Biobank is a large resource of volunteers aged 49-86 years of age at recruitment.[10] Recruitment began in 2006 with follow-up intended for at least 30-years. SARS-CoV-2 test information, ICD-10 hospital codes, death records and general practice prescription information were obtained via the UK Biobank data portal on 13th December 2021. This information covered hospital diagnoses between 18th April 1991 and 30th September 2021, SARS-CoV-2 tests between 13th January 2020 and 18th October 2021, and death records until 12th November 2021. Illustrated in Figure 1 there were 464,306 participants, of whom 4,469 were removed owing to not having a BMI measure or Townsend index score or smoking status and a further 79,457 were removed owing to lack of prescription data. General practice prescription data from 1st of January 2019 through to 27th September 2021, available for 380,380 participants, were used to identify people prescribed methotrexate, folic acid, anticonvulsants (phenytoin, carbamazepine, phenobarbital), iron supplements (ferrous fumarate, ferrous sulfate, ferrous gluconate), and co-prescribed medications.

### Ethics approval statement

The UK Biobank was undertaken with ethical approval from the North West Multi-Centre Research Ethics Committee of the UK. This study was done under this ethical approval; researchers using the UK Biobank do not require separate ethical approval. The study complies with the Declaration of Helsinki and written informed consent was obtained from all participants.

### Patient and public involvement

We did not involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

COVID-19 definitions

The criteria for COVID-19 diagnosis were defined as participants with 1) a positive SARS-CoV-2 PCR test and / or 2) ICD-10 code for confirmed COVID-19 (U07.1) or probable COVID-19 (U07.2) in hospital records, or death records. There were 26,033 cases, of whom 820 died with COVID-19. Figure 2 summarizes how cases were diagnosed.

Ethnicity, age and comorbidity data

Self-reported ethnicity was grouped into White British (British, Irish, White, any other White background), Black British (African, White and Black African, Black or Black British, Caribbean, White and Black Caribbean, Any other Black background), Asian British (Asian or Asian British, Chinese, Indian, Pakistani, Bangladeshi, White and Asian, Any other Asian background), and Other (Other ethnic group, Mixed, Any other mixed background, Do not know, Prefer not to answer). Age was calculated for 2020 from year of birth. Age groups used in the analysis were <60 years (n= 69,849), 60-69 years (n= 120,013), 70-74 years (n= 90,627) and >74 years (n= 99,891). The ICD-10 hospital codes used to determine additional comorbidity status were rheumatoid arthritis (M05), and sickle cell disease (D57). Demographic characteristics of the study population are presented in Table 1.

Statistical analysis

All association analyses in this case-control study were done using R v4.0.2 in RStudio 1.2.5019. Statistical model 1 was adjusted for age group (4 categories), sex, ethnicity, Townsend deprivation index, BMI, smoking status. Model 2 is Model 1 plus adjustment for the presence of rheumatoid arthritis, sickle cell disease (where daily folic acid is prescribed [2]), prescription of statins, prescription of

anticonvulsants (where co-prescription of folic acid often occurs [11]) and iron supplements (supplementary iron has been associated with poorer outcomes of infectious disease, including COVID-19 [12 13]). For methotrexate and folate use, a single variable with 4 levels was used for statistical modeling (no methotrexate or folate, methotrexate only, folate only, methotrexate and folate). Sex-stratified analyses were done using the same approach to explore any differential association with COVID-19 diagnosis or associated mortality. A $p < 0.05$ threshold indicated nominal evidence for association.

**Results**

Study population

Demographic characteristics of the study population are presented in Table 1. The proportion of those diagnosed with COVID-19 while taking methotrexate was similar to the general study population (1.14% vs 1.09%, respectively) although there was a higher proportion of methotrexate prescriptions in the group that died with COVID-19 (1.34%). There was both a higher proportion of those prescribed folic acid who were diagnosed with COVID-19 (5.99% vs 4.31% in the general population) and those prescribed folic acid in those who died with COVID-19 (15.97% vs 4.31% in the general population). Medications co-prescribed with folic acid were investigated (Table S1). Atorvastatin was co-prescribed 23.21% of the time and Simvastatin 9.49% of the time. Due to these high prescription rates and reports describing an association between statin use and reduced mortality from COVID-19 [14 15] we included statins in Model 2.

**Table 1. Study population in the UK Biobank, restricted to those with data on prescriptions**

| Demographic | All N= 380,380 | COVID-19 diagnosis N= 26,033 | No COVID-19 diagnosis N= 354,347 | COVID-19-related death N= 820 | No COVID-19-related death N= 379,560 |
|---|---|---|---|---|---|
| <60 years of age, n(%) | 69,849 ( 18.36 ) | 7,937 ( 30.49 ) | 61,912 ( 17.47 ) | 26 ( 3.17 ) | 69,823 ( 18.4 ) |
| 60-70 years of age, n(%) | 120,013 ( 31.55 ) | 8,568 ( 32.91 ) | 111,445 ( 31.45 ) | 129 ( 15.73 ) | 119,884 ( 31.58 ) |
| 70-75 years of age, n(%) | 90,627 ( 23.83 ) | 4,569 ( 17.55 ) | 86,058 ( 24.29 ) | 171 ( 20.85 ) | 90,456 ( 23.83 ) |
| >75 years of age, n(%) | 99,891 ( 26.26 ) | 4,959 ( 19.05 ) | 94,932 ( 26.79 ) | 494 ( 60.24 ) | 99,397 ( 26.19 ) |
| Female sex, n(%) | 211,363 ( 55.57 ) | 13,802 ( 53.02 ) | 197,561 ( 55.75 ) | 286 ( 34.88 ) | 211,077 ( 55.61 ) |
| White British, n(%) | 357,620 ( 94.02 ) | 23,807 ( 91.45 ) | 333,813 ( 94.21 ) | 744 ( 90.73 ) | 356,876 ( 94.02 ) |
| Black British, n(%) | 9,826 ( 2.58 ) | 1,021 ( 3.92 ) | 8,805 ( 2.48 ) | 36 ( 4.39 ) | 9,790 ( 2.58 ) |
| Asian British, n(%) | 7,329 ( 1.93 ) | 732 ( 2.81 ) | 6,597 ( 1.86 ) | 25 ( 3.05 ) | 7,304 ( 1.92 ) |
| Other ethnicity, n(%) | 5,605 ( 1.47 ) | 473 ( 1.82 ) | 5,132 ( 1.45 ) | 15 ( 1.83 ) | 5,590 ( 1.47 ) |
| Prescribed methotrexate only, n(%) | 174 ( 0.05 ) | 11 ( 0.04 ) | 163 ( 0.05 ) | 0 ( 0 ) | 174 ( 0.05 ) |
| Prescribed folic acid only, n(%) | 12,433 ( 3.27 ) | 1,273 ( 4.89 ) | 11,160 ( 3.15 ) | 120 ( 14.63 ) | 12,313 ( 3.24 ) |
| Prescribed methotrexate and folic acid, n(%) | 3,952 ( 1.04 ) | 287 ( 1.1 ) | 3,665 ( 1.03 ) | 11 ( 1.34 ) | 3,941 ( 1.04 ) |
| Prescribed neither methotrexate nor folic acid, n(%) | 363,821 ( 95.65 ) | 24,462 ( 93.97 ) | 339,359 ( 95.77 ) | 689 ( 84.02 ) | 363,132 ( 95.67 ) |
| Rheumatoid arthritis, n(%) | 999 ( 0.26 ) | 97 ( 0.37 ) | 902 ( 0.25 ) | 8 ( 0.98 ) | 991 ( 0.26 ) |
| Sickle cell disease, n(%) | 517 ( 0.14 ) | 51 ( 0.2 ) | 466 ( 0.13 ) | 2 ( 0.24 ) | 515 ( 0.14 ) |
| Prescribed anticonvulsant medication, n(%) | 1,642 ( 0.43 ) | 120 ( 0.46 ) | 1,522 ( 0.43 ) | 10 ( 1.22 ) | 1,632 ( 0.43 ) |
| Prescribed statins, n(%) | 156,064 ( 41.03 ) | 10,398 ( 39.94 ) | 145,666 ( 41.11 ) | 521 ( 63.54 ) | 155,543 ( 40.98 ) |
| Prescribed iron supplements, n(%) | 18,471 ( 4.86 ) | 1,661 ( 6.38 ) | 16,810 ( 4.74 ) | 106 ( 12.93 ) | 18,365 ( 4.84 ) |
| BMI, mean(sd) | 27.41 ( 4.76 ) | 28.12 ( 5.03 ) | 27.35 ( 4.74 ) | 30.21 ( 5.79 ) | 27.4 ( 4.76 ) |
| Townsend deprivation index, mean (sd) | -1.35 ( 3.04 ) | -0.9 ( 3.18 ) | -1.39 ( 3.03 ) | -0.28 ( 3.4 ) | -1.36 ( 3.04 ) |
| Never smoked, n(%) | 210,993 ( 55.47 ) | 13,990 ( 53.74 ) | 197,003 ( 55.6 ) | 321 ( 39.15 ) | 210,672 ( 55.5 ) |
| Current smoker, n(%) | 132,222 ( 34.76 ) | 9,262 ( 35.58 ) | 122,960 ( 34.7 ) | 384 ( 46.83 ) | 131,838 ( 34.73 ) |
| Former smoker, n(%) | 37,165 ( 9.77 ) | 2,781 ( 10.68 ) | 34,384 ( 9.7 ) | 115 ( 14.02 ) | 37,050 ( 9.76 ) |

Association with a diagnosis of COVID-19

Compared with people prescribed neither folic acid nor methotrexate, individuals prescribed folic acid had significantly increased risk of diagnosis of COVID-19 in Model 1 (OR 1.60 [1.50 ; 1.70]) (Table 2). In Model 2, which included a diagnosis of RA, sickle cell disease, and prescription of anticonvulsants or statins or iron supplements, this association was not attenuated (OR 1.51 [1.42 ; 1.61]). The prescription of methotrexate without folic acid was uncommon (only 174 people) and did not show an association with COVID-19 diagnosis in either Model. The prescription of methotrexate in combination with folic acid was associated with an increased risk for a diagnosis of COVID-19 in Model 1 (1.15 [ 1.02 ; 1.30 ]) but not in Model 2 (1.09 [0.96 ; 1.23 ]) (Table 2 and Table S2). The risk for COVID-19 diagnosis was associated with similar magnitudes with the prescription of folic acid in men and women in Model 2 (OR 1.50 [1.37 ; 1.64] and 1.52 [1.39 ; 1.65], respectively) (Table S3). The Model 2 sex-specific associations were not statistically significant and of similar magnitudes with methotrexate and with methotrexate combined with folic acid.

**Table 2. COVID-19 diagnosis in people prescribed methotrexate and / or folic acid in the UKBB, compared to people not prescribed methotrexate or folic acid**

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Neither Folic acid nor Methotrexate N= 363,821 | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only N= 12,433 | 1.58 [1.49 ; 1.68 ] | <0.001 | 1.60 [1.50 ; 1.70] | <0.001 | 1.51 [1.42 ; 1.61] | <0.001 |
| Methotrexate only N=174 | 0.94 [0.51 ; 1.72] | 0.83 | 0.89 [0.48 ; 1.65] | 0.72 | 0.86 [0.47 ; 1.6] | 0.64 |
| Methotrexate and Folic acid N= 3,952 | 1.09 [0.96 ; 1.23] | 0.18 | 1.15 [1.02 ; 1.30] | 0.021 | 1.09 [0.96 ; 1.23] | 0.18 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status.

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Association with mortality related to a COVID-19 diagnosis

In the general population, compared with people prescribed neither folic acid nor methotrexate, individuals prescribed folic acid had a significant association with mortality related to COVID-19 in Model 1 (OR 2.91 [2.38 ; 3.55]) (Table 3 and Table S4). In Model 2, which included a diagnosis of RA, sickle cell

disease, prescription of anticonvulsants, statins and iron supplements, this association was maintained (OR 2.64 [2.15 ; 3.24]). Although there was a higher proportion of methotrexate prescriptions in the group that died with COVID-19, there were no deaths reported in individuals diagnosed with COVID-19 who were prescribed only methotrexate (N = 11). Moreover, the prescription of methotrexate in combination with folic acid was not associated with an increased odds for death after diagnosis of COVID-19 in Model 1 (Table 3) (1.26 [ 0.70 ; 2.30 ]) or Model 2 (1.07 [0.57 ; 1.98 ]). The risk for mortality after COVID-19 diagnosis was of similar magnitude with the prescription of folic acid in both men and women in Model 2 (OR 2.59 [2.00 ; 3.36] and 2.72 [1.93 ; 3.84], respectively) (Table S5). In both men and women co-prescription of methotrexate attenuated the association.

**Table 3. The association of prescription of methotrexate and folic acid with COVID-19-related death in the UK Biobank***

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither Folic acid nor Methotrexate N= 363,821 | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only N= 12,433 | 5.14 [4.23 ; 6.24] | <0.001 | 2.91 [2.38 ; 3.55] | <0.001 | 2.64 [2.15 ; 3.24] | <0.001 |
| Methotrexate and folic acid N= 3,952 | 1.47 [0.81 ; 2.67] | 0.21 | 1.26 [0.70 ; 2.30] | 0.44 | 1.07 [0.57 ; 1.98] | 0.84 |

*There were no deaths in the group of participants taking only methotrexate without folate.

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status.

Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

To account for improvements in outcome of patients with COVID-19, resulting from changes in public health measures and emergence of different SARS-CoV-2 lineages over time [16] we tested for association with death in the COVID-19-positive cohort including also a quarterly (3-monthly) categorical time variable for diagnosis of COVID-19 using Model 2 (Table S6). This revealed a similar pattern of association with death - there was association with increased risk of death in patients prescribed folic acid only (OR 1.46 [1.16 ; 1.83]) but not in the group prescribed both folic acid and methotrexate (OR 0.96 [0.50 ; 1.83]).

**Discussion**

In this population-based analysis, we report association with a 1.5-fold increased risk for COVID-19 diagnosis and 2.6-fold increased risk for COVID-19-related death among those who had been prescribed folic acid supplementation. The prescription of methotrexate was not associated with an increased risk of diagnosis of COVID-19 and we were not able to make an estimate for COVID-19-related death in the small sample of those prescribed methotrexate only. Notably, those prescribed methotrexate and folic acid did not have an increased risk for COVID-19 diagnosis or associated death, indicating that methotrexate might attenuate the increased risk for COVID-19 diagnosis and related-death which were associated with the prescription of folic acid alone.

In the context of SARS-Cov-2 infection it is established that hijacking of cellular metabolic pathways is important for viral replication.[17] Zhang et al described that SARS-CoV-2 remodels host folate and one-carbon metabolism at the post-transcriptional level to support de novo purine synthesis, bypassing viral shutoff of host translation. [8] This suggests that viral replication could be sensitive to folate inhibitors, such as methotrexate. Intracellular glucose and folate are depleted in SARS-CoV-2-infected cells, and viral replication is exquisitely sensitive *in vitro* to inhibitors of folate and one carbon metabolism, notably methotrexate.[8] Stegmann et al, based on cell culture experiments, reported that methotrexate alone or in combination with remdesivir limits the replication of SARS-CoV-2.[18] With the caveat that our study is observational epidemiology and causality cannot be inferred, our study does support the possibility that external folate supply facilitates the production of large amounts of virus, contributing to clinical infection and mortality. With the same caveat on inference of causality from observational data our study also supports the notion that SARS-CoV-2 replication is enhanced by folate supply based on our finding that co-prescription of an antifolate (methotrexate) attenuated the association of supplementation with folic acid with COVID-19 outcomes.

There is also evidence that inadequate folate status may be harmful in the context of host resistance to infection with SARS-CoV-2. In addition to the well-recognized complication of anemia, folate deficiency has other detrimental health effects, including suppression of immune function.[19] Additional support for the possibility that adequate folate status is important in COVID-19 outcomes is provided by the observation that folate deficiency was associated with poorer outcomes in a cohort of COVID-19 patients.[20] It is important to note that it is possible that in the study by Itelman et al,[20] if increased folate levels were causal of COVID-19 diagnosis and poor outcomes, that the association with lower folate levels could have been caused by selection (collider) bias.[21] Vitamin B12 deficiency has also been proposed as a factor related to poor COVID-19 outcomes, presumed to be through the induction of functional folate deficiency.[22] A drug-protein structure interaction analysis raises the possibility that folate blocks the 3CL hydrolase enzyme, which may affect viral entry and replication.[23] It is therefore possible that both inadequate and excessive amounts of folate may be detrimental to host resistance to SARS-CoV-2 infection and that there may be an optimal range of physiological folate status related to host-resistance to COVID-19 infection and severity.

Data from the COVID-19 Global Rheumatology Alliance describes that a number of immunomodulatory drugs used in rheumatology are associated with an increased risk of infection and death compared with methotrexate. [7] Being on no DMARD therapy was associated with an increased risk of death with COVID-19 (OR 2.11 [1.48 ; 3.01]), which could be interpreted as either a protective effect of methotrexate or an increased risk for death associated with poor rheumatic disease control. The authors of the study additionally noted that people not on DMARD therapy had increased use of glucocorticoids meaning that confounding by indication cannot be ruled out as an explanation.[24] Methotrexate was also associated with lower odds for death when compared with sulfasalazine, other immunosuppressants and rituximab. In no case was methotrexate associated with an increased risk for death. The COVID-19 Global Rheumatology Alliance study did not explore the effect of folic acid supplementation in the

setting of methotrexate, although it is it is highly likely that almost all patients on methotrexate also were receiving folic acid supplementation. Considering the widespread use of folic acid supplements and proposals to abandon entirely tolerable upper intake levels for folic acid [25] it would be prudent to monitor the effect of increased folic acid intake at a population level on COVID-19 morbidity and mortality, particularly at the upper end of folic acid intake.

Limitations

Limitations of our analysis are important to note. First, given the small size of the methotrexate-only group and that there were no deaths related to COVID-19 in this group we could not test a beneficial effect on mortality of methotrexate in isolation. It is uncommon to find patients with methotrexate prescribed without supplemental folic acid as this is the standard of care. Second, over the time period of this study (March 2020 – November 2021), COVID-19 outcomes (i.e. death) will have been influenced by the development of clinical treatments including antiviral drugs and monoclonal antibodies, changes to public health measures and the appearance of new COVID-19 strains [16] . We were unable to account for these factors in the population-based analysis however we attempted to account for this in the analysis within the COVID-19-positive group by including a time variable (Table S4). Third, findings are not necessarily generalizable outside of the middle-aged (>45 years of age) white European cohort that dominates the UK Biobank. Fourth, the full extent of SARS-CoV-2 infection is not known in the UK population due to incomplete testing rates early in the pandemic. Fifth, prescription data were single script from General Practitioners only, and it was not possible to ascertain compliance, dosing or frequency of administration, or whether participants were taking the prescribed medication during the COVID-19 pandemic or continuously during the study period although we attempted to account for this by restricting our use of prescription information to the years 2019 and 2020 only. Sixth, although we included rheumatoid arthritis in Model 2, we were unable to account for any potential effect of disease activity in RA in people prescribed folic acid. Rheumatoid arthritis disease activity negatively impacts

death from COVID-19 outcomes.[7] Seventh, while it is a strength of our study that mandatory fortification of the UK diet with folic acid had not been introduced during the period of our study and thus did not confound our analysis, we were unable to account for the lower-dose over the counter folic acid supplementation available in the UK (400 micrograms being the most common formulation for over the counter tablets) because there were no self-report information in the UK Biobank dataset on the use of folic acid supplementation. Finally, residual confounding conferred by the underlying indications for folate prescriptions (besides the ones addressed in our analysis) is a possibility. Finally, given that our study was an observational case-control design, no firm inference can be made for causality of folic acid supplementation on mortality from COVID-19.

## Conclusion

In conclusion, and despite the limitations of our study enumerated above, our data support the hypothesis that increased folate resulting from pharmacologic folic acid prescription could contribute to a higher probability of contracting clinically-detectable infection with SARS-CoV2 and to an increase in the risk of death following the infection. The study population was drawn from the >45 year old segment of the UK population and is predominantly of white European ethnicity, therefore our findings have reduced generalizability to younger people, to other ethnic groups and to other countries. Nevertheless, our findings justify future studies on the influence of folic acid supplementation on COVID-19 outcomes, particularly in pregnant women and people on anticonvulsants requiring supplementary folic acid.

As a final comment, we point out that attention is currently being directed toward establishing whether excessive intake of folate, particularly in the form of folic acid, may have undesirable and potentially deleterious effects.[26] The possibility that susceptibility to COVID-19 infection and its serious and even

fatal complications may be affected by folic acid intake and folate status should be thoroughly investigated.

**Contributors**

RKT, RG, SLM, PCR, TRM and ALG substantially contributed to study conception and design, to acquisition and analysis of data and interpretation of results. RKT, RG, SLM, PCR, TRM and ALG contributed to drafting the article and critical revision and RKT, RG, SLM, PCR, TRM and ALG approved the final version. RKT and TRM directly accessed and verified the underlying data reported in the manuscript.

**Funding**

None.

**Competing interests**

Phillip Robinson reports personal fees from Abbvie, Atom Biosciences, Eli Lilly, Gilead, Janssen, Novartis, UCB, Roche, Pfizer; meeting attendance support from BMS, Pfizer and UCB Pharma and grant funding from Janssen, Novartis, Pfizer and UCB Pharma, all outside the submitted work. Angelo Gaffo reports personal fees from SOBI, Selecta and honoraria from UptoDate, Inc. outside the submitted work. All other authors have no declarations of interest.

**Ethics approval**

The UK Biobank was undertaken with ethical approval from the North West Multi-Centre Research Ethics Committee of the UK. This study was done under this ethical approval; researchers using the UK Biobank do not require separate ethical approval. The study complies with the Declaration of Helsinki and written informed consent was obtained from all participants.

**Data availability statement**

Data are available in a public, open access repository.

**References**


1. Department of Health and Social Care UG. [Available from: https://www.gov.uk/government/news/folic-acid-added-to-flour-to-prevent-spinal-conditions-in-babies accessed 1/11 2022.
2. Dixit R, Nettem S, Madan SS, et al. Folate supplementation in people with sickle cell disease. *Cochrane Database Syst Rev* 2018;3:CD011130. doi: 10.1002/14651858.CD011130.pub3 [published Online First: 2018/03/17]
3. Linnebank M, Moskau S, Semmler A, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol* 2011;69(2):352-9. doi: 10.1002/ana.22229 [published Online First: 2011/01/20]
4. Fraenkel L, Bathon JM, England BR, et al. 2021 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol* 2021;73(7):1108-23. doi: 10.1002/art.41752 [published Online First: 2021/06/09]
5. Morgan SL, Baggott JE, Vaughn WH, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med* 1994;121(11):833-41. doi: 10.7326/0003-4819-121-11-199412010-00002 [published Online First: 1994/12/01]
6. Morgan SL, Baggott JE, Vaughn WH, et al. The effect of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 1990;33(1):9-18. doi: 10.1002/art.1780330102 [published Online First: 1990/01/01]
7. Strangfeld A, Schäfer M, Gianfrancesco MA, et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Annals of the Rheumatic Diseases* 2021;80(7):930-42. doi: 10.1136/annrheumdis-2020-219498
8. Zhang Y, Guo R, Kim SH, et al. SARS-CoV-2 hijacks folate and one-carbon metabolism for viral replication. *Nature Communications* 2021;12(1):1676. doi: 10.1038/s41467-021-21903-z
[Dataset] 9. [Available from: https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access accessed June 22 2022.
10. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12(3):e1001779. doi: 10.1371/journal.pmed.1001779 [published Online First: 2015/04/01]
11. Moore JL. The significance of folic acid for epilepsy patients. *Epilepsy Behav* 2005;7(2):172-81. doi: 10.1016/j.yebeh.2005.05.020 [published Online First: 2005/08/02]
12. Girelli D, Marchi G, Busti F, et al. Iron metabolism in infections: Focus on COVID-19. *Semin Hematol* 2021;58(3):182-87. doi: 10.1053/j.seminhematol.2021.07.001 [published Online First: 2021/08/15]
13. Gonciarz RL, Renslo AR. Emerging role of ferrous iron in bacterial growth and host-pathogen interaction: New tools for chemical (micro)biology and antibacterial therapy. *Curr Opin Chem Biol* 2021;61:170-78. doi: 10.1016/j.cbpa.2021.01.015 [published Online First: 2021/03/15]
14. Diaz-Arocutipa C, Melgar-Talavera B, Alvarado-Yarasca A, et al. Statins reduce mortality in patients with COVID-19: an updated meta-analysis of 147 824 patients. *Int J Infect Dis* 2021;110:374-81. doi: 10.1016/j.ijid.2021.08.004 [published Online First: 2021/08/11]
15. Kollias A, Kyriakoulis KG, Kyriakoulis IG, et al. Statin use and mortality in COVID-19 patients: Updated systematic review and meta-analysis. *Atherosclerosis* 2021;330:114-21. doi: 10.1016/j.atherosclerosis.2021.06.911 [published Online First: 2021/07/11]

16. Davies NG, Jarvis CI, Group CC-W, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature* 2021;593(7858):270-74. doi: 10.1038/s41586-021-03426-1 [published Online First: 2021/03/17]
17. Thaker SK, Ch'ng J, Christofk HR. Viral hijacking of cellular metabolism. *BMC Biology* 2019;17(1):59. doi: 10.1186/s12915-019-0678-9
18. Stegmann KM, Dickmanns A, Gerber S, et al. The folate antagonist methotrexate diminishes replication of the coronavirus SARS-CoV-2 and enhances the antiviral efficacy of remdesivir in cell culture models. *Virus Res* 2021;302:198469. doi: 10.1016/j.virusres.2021.198469 [published Online First: 2021/06/07]
19. Green R, Miller JW. Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations of dysfunctional folate status. *Semin Hematol* 1999;36(1):47-64. [published Online First: 1999/02/04]
20. Itelman E, Wasserstrum Y, Segev A, et al. Clinical Characterization of 162 COVID-19 patients in Israel: Preliminary Report from a Large Tertiary Center. *Isr Med Assoc J* 2020;22(5):271-74. [published Online First: 2020/05/08]
21. Choi HK, Nguyen US, Niu J, et al. Selection bias in rheumatic disease research. *Nat Rev Rheumatol* 2014;10(7):403-12. doi: 10.1038/nrrheum.2014.36 [published Online First: 2014/04/02]
22. Wee AKH. COVID-19's toll on the elderly and those with diabetes mellitus - Is vitamin B12 deficiency an accomplice? *Med Hypotheses* 2021;146:110374. doi: 10.1016/j.mehy.2020.110374 [published Online First: 2020/12/02]
23. Serseg T, Benarous K, Yousfi M. Hispidin and Lepidine E: Two Natural Compounds and Folic Acid as Potential Inhibitors of 2019-novel Coronavirus Main Protease (2019- nCoVM(pro)), Molecular Docking and SAR Study. *Curr Comput Aided Drug Des* 2021;17(3):469-79. doi: 10.2174/1573409916666200422075440 [published Online First: 2020/04/24]
24. Schafer M, Strangfeld A, Hyrich KL, et al. Response to: 'Correspondence on 'Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician reported registry'' by Mulhearn et al. *Ann Rheum Dis* 2021 doi: 10.1136/annrheumdis-2021-220134 [published Online First: 2021/03/03]
25. Wald NJ, Morris JK, Blakemore C. Public health failure in the prevention of neural tube defects: time to abandon the tolerable upper intake level of folate. *Public Health Rev* 2018;39:2. doi: 10.1186/s40985-018-0079-6 [published Online First: 2018/02/17]
26. Maruvada P, Stover PJ, Mason JB, et al. Knowledge gaps in understanding the metabolic and clinical effects of excess folates/folic acid: a summary, and perspectives, from an NIH workshop. *Am J Clin Nutr* 2020;112(5):1390-403. doi: 10.1093/ajcn/nqaa259 [published Online First: 2020/10/07]

**Figure legends**

**Figure 1. Flow schematic of study design**

**Figure 2. Data sources of COVID-19-diagnosed individuals**

Of the 26,033 COVID-19-diagnosed individuals, 25,300 were identified from positive SARS-Cov2 test results (21,705 unique to this group), 4,170 identified from hospital records (650 unique to this group), and 820 identified from death records (51 unique to this group). 217 diagnosed after 13th October 2021 (30 days before the last recorded death) were removed from the case only analysis in Table S4 given the unknown outcome.

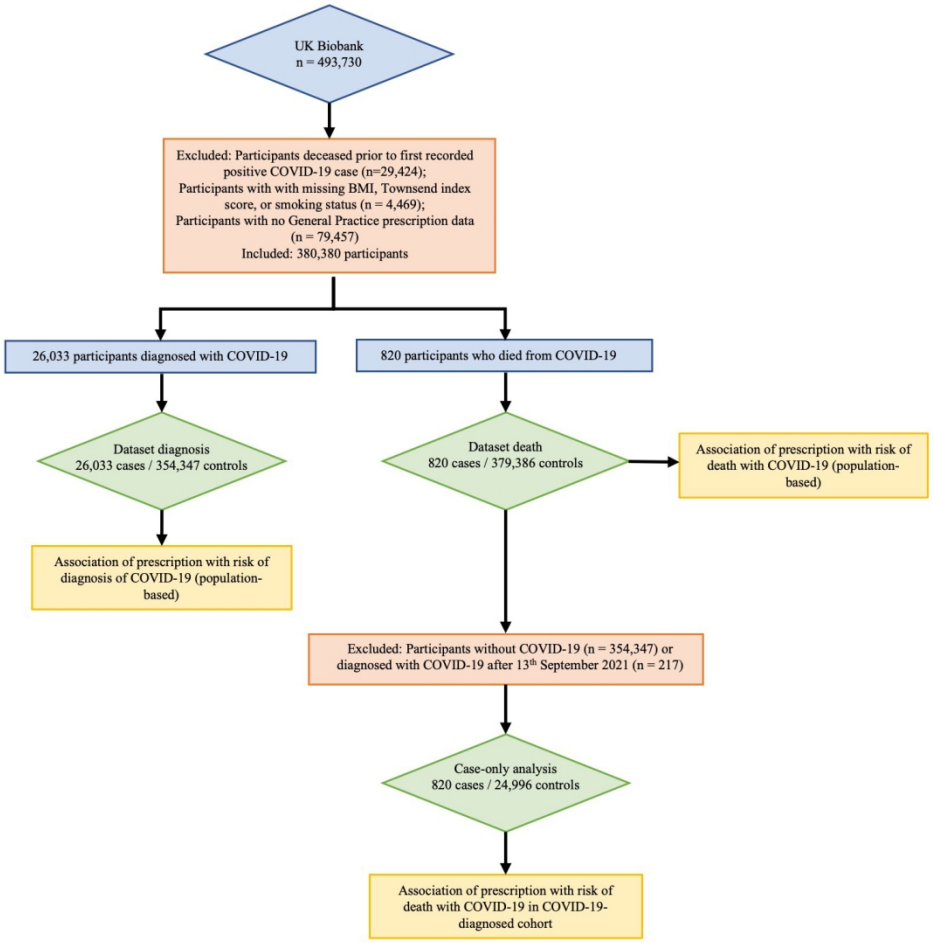


Figure 1 Flow schematic of study design

181x190mm (225 x 225 DPI)

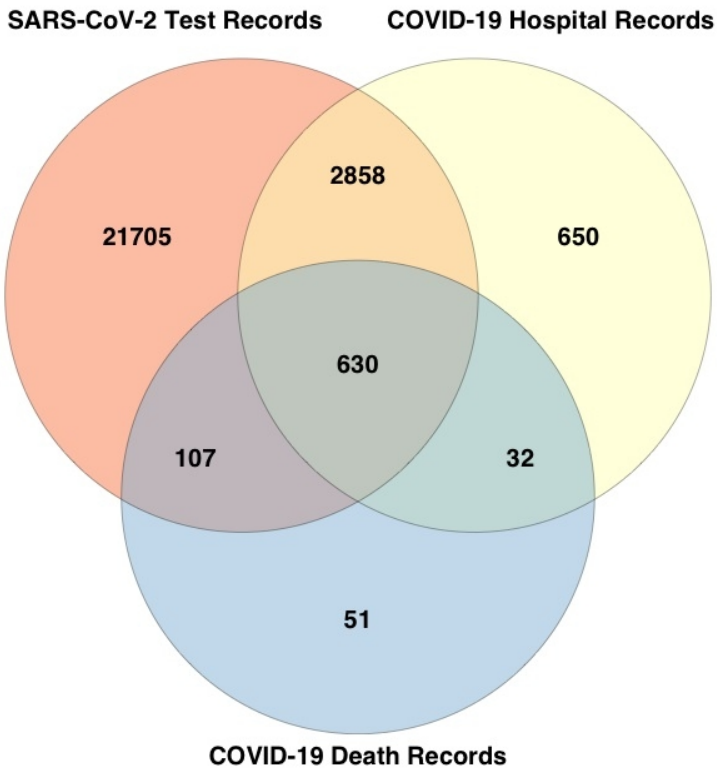


Figure 2 Data sources of COVID-19-diagnosed individuals. Of the 26,033 COVID-19-diagnosed individuals, 25,300 were identified from positive SARS-Cov2 test results (21,705 unique to this group), 4,170 identified from hospital records (650 unique to this group), and 820 identified from death records (51 unique to this group). 217 diagnosed after 13th October 2021 (30 days before the last recorded death) were removed from the case only analysis in Table S4 given the unknown outcome.

211x211mm (96 x 96 DPI)

Table S1. Medications co-prescribed with folic acid on >5% of occasions

| Medication/Supplement | Number of prescriptions* | Percentage of folic acid prescriptions |
|---|---|---|
| Folic acid | 177,918 | 100.00 |
| Methotrexate | 45,697 | 25.68 |
| Atorvastatin | 41,296 | 23.21 |
| Lansoprazole | 35,611 | 20.02 |
| Omeprazole | 32,909 | 18.50 |
| Bisoprolol | 26,445 | 14.86 |
| Levothyroxine | 25,482 | 14.32 |
| Ramipril | 21,990 | 12.36 |
| Aspirin | 21,696 | 12.19 |
| Paracetamol | 20,830 | 11.71 |
| Amlodipine | 18,354 | 10.32 |
| Simvastatin | 16,878 | 9.49 |
| Amitriptyline | 13,573 | 7.63 |
| Furosemide | 12,983 | 7.30 |
| Metformin | 11,460 | 6.44 |
| Hydroxychloroquine | 11,038 | 6.20 |
| Clopidogrel | 10,918 | 6.14 |
| Prednisolone | 10,475 | 5.89 |
| Alendronic acid | 10,198 | 5.73 |

*General practice prescription data from 1st of January 2019 through to 27th September 2021

Table S2. COVID-19 diagnosis in people prescribed methotrexate and / or folic acid in the UKBB, compared to people not prescribed methotrexate or folic acid

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Prescribed folic acid only[1] | 1.58 [1.49 ; 1.68 ] | <0.001 | 1.60 [ 1.5 ; 1.7 ] | <0.001 | 1.51 [ 1.42 ; 1.61 ] | <0.001 |
| Prescribed methotrexate only[1] | 0.94 [0.51 ; 1.72] | 0.83 | 0.89 [ 0.48 ; 1.65 ] | 0.72 | 0.86 [ 0.47 ; 1.6 ] | 0.64 |
| Prescribed methotrexate and folic acid[1] | 1.09 [0.96 ; 1.23] | 0.18 | 1.15 [ 1.02 ; 1.3 ] | 0.021 | 1.09 [ 0.96 ; 1.23 ] | 0.18 |
| 60-70 years of age[2] | | | 0.60 [ 0.58 ; 0.62 ] | <0.001 | 0.59 [ 0.57 ; 0.61 ] | <0.001 |
| 70-75 years of age[2] | | | 0.41 [ 0.4 ; 0.43 ] | <0.001 | 0.40 [ 0.38 ; 0.41 ] | <0.001 |
| >75 years of age[2] | | | 0.40 [ 0.38 ; 0.41 ] | <0.001 | 0.38 [ 0.37 ; 0.4 ] | <0.001 |
| Male sex | | | 1.09 [ 1.07 ; 1.12 ] | <0.001 | 1.09 [ 1.06 ; 1.12 ] | <0.001 |
| Former smoker[3] | | | 0.87 [ 0.84 ; 0.92 ] | <0.001 | 0.87 [ 0.83 ; 0.91 ] | <0.001 |
| Never smoked[3] | | | 0.88 [ 0.86 ; 0.91 ] | <0.001 | 0.89 [ 0.86 ; 0.91 ] | <0.001 |
| BMI | | | 1.03 [ 1.03 ; 1.03 ] | <0.001 | 1.03 [ 1.03 ; 1.03 ] | <0.001 |
| Asian British[4] | | | 1.38 [ 1.29 ; 1.48 ] | <0.001 | 1.33 [ 1.25 ; 1.43 ] | <0.001 |
| Black British[4] | | | 1.08 [ 1 ; 1.17 ] | 0.057 | 1.08 [ 1 ; 1.18 ] | 0.058 |
| Other ethnicity[4] | | | 1.05 [ 0.95 ; 1.15 ] | 0.35 | 1.04 [ 0.95 ; 1.15 ] | 0.40 |
| Townsend deprivation index | | | 1.03 [ 1.02 ; 1.03 ] | <0.001 | 1.03 [ 1.02 ; 1.03 ] | <0.001 |
| Rheumatoid arthritis | | | | | 1.4 [ 1.12 ; 1.74 ] | 0.003 |
| Sickle cell disease | | | | | 1.00 [ 0.74 ; 1.35 ] | 1.00 |
| Prescribed anticonvulsant medication | | | | | 1.07 [ 0.89 ; 1.29 ] | 0.48 |
| Prescribed statins | | | | | 1.08 [ 1.05 ; 1.12 ] | <0.001 |
| Prescribed iron supplements | | | | | 1.29 [ 1.22 ; 1.36 ] | <0.001 |

1 Compared to Prescribed neither methotrexate nor folic acid; 2 Compared to <60 years of age; Compared to Current smoker; 4 Compared to White British; Model 1 and Model 2 contain all variables with odds ratios present (and comparative groups) as respectively listed below the titles.

Table S3. Sex-specific associations between prescription of methotrexate, folic acid, and the combination of both with the risk of COVID-19

| | COVID-19 diagnosis - Male | | | | | | COVID-19 diagnosis -Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | | *Model 1* | | *Model 2* | | *Unadjusted* | | *Model 1* | | *Model 2* | |
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | OR, [95% CI] | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 1.52 [ 1.39 ; 1.66 ] | <0.001 | 1.57 [ 1.44 ; 1.72 ] | <0.001 | 1.50 [ 1.37 ; 1.64 ] | <0.001 | 1.64 [ 1.51 ; 1.77 ] | <0.001 | 1.61 [ 1.48 ; 1.75 ] | <0.001 | 1.52 [ 1.39 ; 1.65 ] | <0.001 |
| Methotrexate only | 0.96 [ 0.39 ; 2.38 ] | 0.93 | 0.93 [ 0.37 ; 2.32 ] | 0.88 | 0.92 [ 0.37 ; 2.30 ] | 0.86 | 0.92 [ 0.4 ; 2.11 ] | 0.85 | 0.87 [ 0.38 ; 1.99 ] | 0.74 | 0.83 [ 0.36 ; 1.90 ] | 0.65 |
| Methotrexate and folic acid | 1.08 [ 0.89 ; 1.31 ] | 0.45 | 1.15 [ 0.94 ; 1.40 ] | 0.17 | 1.11 [ 0.91 ; 1.36 ] | 0.29 | 1.11 [ 0.95 ; 1.29 ] | 0.19 | 1.16 [ 0.99 ; 1.36 ] | 0.060 | 1.07 [ 0.91 ; 1.26 ] | 0.38 |

diagnosis

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status
Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Table S4. The association of prescription of methotrexate and folic acid with COVID-19-related death in the UK Biobank.

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Prescribed folic acid only[1] | 1.47 [0.81 ; 0.67] | 0.21 | 2.91 [ 2.38 ; 3.55 ] | <0.001 | 2.64 [ 2.15 ; 3.24 ] | <0.001 |
| Prescribed methotrexate only[1] | N/A | N/A | N/A | N/A | N/A | N/A |
| Prescribed methotrexate and folic acid[1] | 5.14 [4.23 ; 6.24] | <0.001 | 1.26 [ 0.7 ; 2.3 ] | 0.44 | 1.07 [ 0.57 ; 1.98 ] | 0.84 |
| 60-70 years of age[2] | | | 3.05 [ 2 ; 4.65 ] | <0.001 | 2.98 [ 1.95 ; 4.56 ] | <0.001 |
| 70-75 years of age[2] | | | 5.70 [ 3.76 ; 8.64 ] | <0.001 | 5.46 [ 3.59 ; 8.31 ] | <0.001 |
| >75 years of age[2] | | | 14.7 [ 9.86 ; 21.91 ] | <0.001 | 13.87 [ 9.26 ; 20.78 ] | <0.001 |
| Male sex | | | 2.13 [ 1.84 ; 2.46 ] | <0.001 | 2.13 [ 1.84 ; 2.47 ] | <0.001 |
| Former smoker[3] | | | 1.37 [ 1.11 ; 1.7 ] | 0.004 | 1.37 [ 1.11 ; 1.7 ] | 0.004 |
| Never smoked[3] | | | 0.76 [ 0.65 ; 0.89 ] | <0.001 | 0.77 [ 0.66 ; 0.89 ] | 0.001 |
| BMI | | | 1.09 [ 1.08 ; 1.11 ] | <0.001 | 1.09 [ 1.08 ; 1.11 ] | <0.001 |
| Asian British[4] | | | 2.48 [ 1.76 ; 3.49 ] | <0.001 | 2.33 [ 1.65 ; 3.29 ] | <0.001 |
| Black British[4] | | | 1.74 [ 1.15 ; 2.63 ] | <0.001 | 1.72 [ 1.13 ; 2.63 ] | 0.012 |
| Other ethnicity[4] | | | 1.44 [ 0.86 ; 2.42 ] | 0.16 | 1.44 [ 0.86 ; 2.41 ] | 0.17 |
| Townsend deprivation index | | | 1.09 [ 1.07 ; 1.11 ] | <0.001 | 1.08 [ 1.06 ; 1.11 ] | <0.001 |
| Rheumatoid arthritis | | | | | 2.48 [ 1.19 ; 5.17 ] | 0.015 |
| Sickle cell disease | | | | | 1.22 [ 0.29 ; 5.15 ] | 0.78 |
| Prescribed anticonvulsant medication | | | | | 2.22 [ 1.18 ; 4.17 ] | 0.014 |
| Prescribed statins | | | | | 1.08 [ 0.93 ; 1.25 ] | 0.33 |
| Prescribed iron supplements | | | | | 1.44 [ 1.16 ; 1.79 ] | 0.001 |

1 Compared to Prescribed neither methotrexate nor folic acid; 2 Compared to <60 years of age; Compared to Current smoker; 4 Compared to White British; N/A = not applicable due to no deaths in the methotrexate only group. Model 1 and Model 2 contain all variables with odds ratios present (and comparative groups) as respectively listed below the titles.

Table S5. Sex-specific associations between prescription of methotrexate, folic acid, and the combination of both with the risk of COVID-19 related mortality.

| | COVID-19 related death – Male | | | | | | COVID-19 related death - Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | | *Model 1* | | *Model 2* | | *Unadjusted* | | *Model 1* | | *Model 2* | |
| | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | OR, [95% CI] | OR, [95% CI] | *P* | OR, [95% CI] | *P* | OR, [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 4.73 [3.71;6.04] | <0.001 | 2.83 [2.20;3.64 ] | <0.001 | 2.59 [2.00;3.36] | <0.001 | 5.67 [4.10;7.82 ] | <0.001 | 3.07 [2.20; 4.28] | <0.001 | 2.72 [1.93;3.84] | <0.001 |
| Methotrexate and folic acid | 2.00 [0.99;4.03] | 0.053 | 1.62 [0.80;3.26] | 0.18 | 1.54 [0.75;3.15] | 0.24 | 1.01 [0.32;3.16] | 0.99 | 0.80 [0.25;2.50] | 0.70 | 0.56 [0.17;1.84] | 0.34 |

Model 1 adjusted for age group, sex, ethnicity, Townsend deprivation index, BMI, smoking status
Model 2 is model 1 plus adjustment by the presence of rheumatoid arthritis, sickle cell disease, use of statins, anticonvulsants and iron supplementation.

Table S6. Risk of death related to COVID-19 in the COVID-19-positive group

| | *Unadjusted* | | *Model 1* | | *Model 2* | |
|---|---|---|---|---|---|---|
| | OR [95% CI] | *P* | OR [95% CI] | *P* | OR [95% CI] | *P* |
| Neither folic acid nor methotrexate | 1.0 | - | 1.0 | - | 1.0 | - |
| Folic acid only | 3.58 [ 2.93 ; 4.39 ] | <0.001 | 1.82 [ 1.47 ; 2.26 ] | <0.001 | 1.46 [ 1.16 ; 1.83 ] | 0.0011 |
| Methotrexate and folic acid | 1.37 [ 0.75 ; 2.52 ] | 0.31 | 1.03 [ 0.55 ; 1.91 ] | 0.94 | 0.96 [ 0.50 ; 1.83 ] | 0.90 |

Model 2 additionally adjusted by quarterly categorical time variable.

A total of 25,816 COVID-19-positive cases were analyzed. 217 COVID-19-positive cases who were diagnosed after 13th September 2021 (28 days before the last recorded death) were removed from the cohort given the unknown outcome in these individuals

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 3 | 'A population-based cohort' in third line of the abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 3 | Throughout abstract |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 6-7 | All text in Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 7 | Last sentence of Introduction. |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | 3,7 | Refer items 1a, 1b and 3 above. |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-9 | Throughout the methods |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 8-9 (case-control) | This information is in the Methods sections 'COVID-19 definitions' and 'Prescription data' |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | | Matching was not done in this study. |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 8 | This information is in the Methods section 'Ethnicity, age and comorbidity data'. |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 8-9 | This information is in the Methods sections 'Gout and COVID-19 definitions and case- |

| | | | | |
|---|---|---|---|---|
| | | | | control datasets' and 'Ethnicity, age and comorbidity data'. |
| Bias | 9 | Describe any efforts to address potential sources of bias | Table S4 | This analysis including a categorical time variable was done to account for improvements in treatment over time |
| Study size | 10 | Explain how the study size was arrived at | | The study size comprised all available data from the UK Biobank. |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | | Quantitative variables were BMI, age and Townsend deprivation index. These were all included as potential confounding variables. Age groupings described in the final paragraph on page 6. |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 9 | Refer to Methods section 'Statistical analysis'. |
| | | (*b*) Describe any methods used to examine subgroups and interactions | | The COVID-19 case-only analysis was one subgroup (Table S4). Additional subgroups were men and women. |
| | | (*c*) Explain how missing data were addressed | 8 | Paragraph 1: "there were 464,306 participants, of whom 4,469 were removed owing to not having a BMI measure or Townsend index score or smoking status and a further 112,466 were removed owing to lack of prescription data." |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | | Not applicable |
| | | (*e*) Describe any sensitivity analyses | | No sensitivity analyses were done. |
| **Results** | | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | | Refer Figure 1. |
| | | (b) Give reasons for non-participation at each stage | 8 | Refer paragraph 1. |
| | | (c) Consider use of a flow diagram | | Figure 1 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 18-19 | Table 1 |

| | | | | |
|---|---|---|---|---|
| | | (b) Indicate number of participants with missing data for each variable of interest | | Not applicable. Participants with missing data were excluded. |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | | Not applicable |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | | Not applicable. |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | 18-19 | Table 1 |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 9-10,20-21 | Unadjusted estimates are provided (Tables 2,3). The reason for inclusion of potential confounders in Model 2 is explained in last paragraph pg 9 and first paragraph pg 10. |
| | | (*b*) Report category boundaries when continuous variables were categorized | | This was done for age: "Age was calculated for 2020 from year of birth. Age groups used in the analysis were <60 years (n= 57,618), 60-69 years (n= 107,140), 70-74 years (n= 85,926) and >74 years (n= 96,687)." |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | | Not applicable |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 23-24,26 | Analyses stratified by sex and vaccination status reported in Tables S2, S3, S5. |
| **Discussion** | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 | First paragraph: "In this population-based analysis, we report 1.7-fold increased risk for COVID-19 diagnosis and 3.2-fold increased risk for COVID-19-related death among those having been prescribed folic acid supplementation. The prescription of methotrexate was not associated with an increased risk of diagnosis of COVID-19" |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15-16 | Eight specific limitations are discussed |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-14 | First three paragraphs of Discussion |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 | Paragraph 2: "findings are not necessarily generalizable outside of the middle-aged (>45 years of age) white European cohort that dominates the UK Biobank." |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 7 | Not applicable |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.