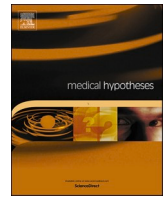




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



COVID-19's toll on the elderly and those with diabetes mellitus – Is vitamin B12 deficiency an accomplice?

Andrew Kien Han Wee^{a, b, *}

^a SingHealth Polyclinics, Marine Parade Polyclinic, Blk 80 Marine Parade Central, #01-792, Singapore 440080, Singapore

^b SingHealth Duke-NUS Medical School, Family Medicine Academic Clinical Programme ("FM ACP"), Office of Academic & Clinical Development, 8 College Road, Singapore 169857, Singapore

ARTICLE INFO

Keywords:

Vitamin B12
 COVID-19
 Diabetes mellitus
 Homocysteine
 One-carbon metabolism
 Folate
 Sars-Cov-2
 Elderly
 Immune system

ABSTRACT

COVID-19 exacts a disproportionate toll on both the elderly and those with diabetes; these patients are more likely to require costly intensive care, longer hospitalisation, and die from complications. Nations would thus find it extremely difficult to either lift or sustain socially, economically, and politically damaging restrictions that keep this group of people safe. Without a vaccine, there is thus an urgent need to identify potential modifiable risk factors which can help manage overall fatality or recovery rates. Case fatality rates are highly variable between (and even within) nations; nutritional differences have been proposed to account significantly for this disparity. Indeed, vitamin B12 deficiency is a common denominator between the elderly and those with diabetes. The question on hand thus lies on whether managing B12 deficiencies will impact COVID-19 fatality outcome or recovery rates. Herein, we review the latest evidence that shows that B12 deficiency associates in multiple areas very similar to where COVID-19 exerts its damaging effects: immunologically; microbiologically; haematologically; and through endothelial cell signalling—supporting the hypothesis that B12 deficiency is a potential modifiable risk factor in our fight against COVID-19.

Introduction

The COVID-19 pandemic exacts a disproportionate toll on the elderly and those with diabetes mellitus (henceforth used interchangeably with “diabetes”); these patients are more likely to require costly intensive care facilities, stay longer in hospital, and die from COVID-19 [1–8]. With currently 703 million persons aged 65 years or older [9] and 422 million people having diabetes mellitus [10] globally, nations worldwide have instituted very costly social restrictions, curfews and lockdowns—especially to keep this vulnerable group of people safe. However, with socioeconomic costs mounting, governments now face the practical dilemma of either sustaining the restrictions or lifting them—with the ever-present risk of resurgent infections re-threatening this vulnerable group and overwhelming healthcare facilities. Indeed,

short of a vaccine, its contagiousness and markedly different clinical presentations [11]—from the asymptomatic to the deadly—make COVID-19 a long-term constant threat.

Major clinical events include: atypical pneumonia; respiratory failure; thromboses; kidney diseases; cerebrovascular and neurological disorders; Kawasaki disease; multi-organ failure; and death [12,13]. Much of the above suggest an underlying cytokine storm [14] and systemic vasculitis [13].

Reported case fatality rates (CFRs) are highly variable. A French study of 1317 hospitalized COVID-19 patients with diabetes mellitus (with an 88.5% predominance of type-2 diabetes mellitus (T2DM)) showed a case fatality of 10.6% (by day 7 of hospitalization); combining both outcomes of “death” and “tracheal intubation for assisted mechanical ventilation” (within 7 days of admission), the prevalence rose

Abbreviations: ACE, Angiotensin-converting enzyme; ALT, Alanine aminotransferase; B12, Vitamin B12; BMI, Body mass index; CFR, Case fatality rate; CI, Confidence intervals; COVID-19, Coronavirus disease caused by SARS-CoV-2; CD4+, T helper cells; CD8+, Cytotoxic T cells; CRP, C-reactive protein; Diabetes, Diabetes mellitus; eGFR, Estimated glomerular filtration rate; HCV, Hepatitis C virus; IL, Interleukin; IFN- γ , Interferon gamma; IRES, Internal ribosomal entry site for the hepatitis C virus; NHANES, National Health And Nutrition Examination Survey; NK cell, Natural killer cell; nsp, Non-structural protein of SARS-CoV-2; OR, Odds ratio; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SD, Standard deviation; SOC, Standard of care; SVR, Sustained viral response; T2DM, Type-2 diabetes mellitus; TNF- α , Tumour necrosis factor alpha; UMFA, Unmetabolized folic acid.

* Address: Marine Parade Polyclinic Blk 80 Marine Parade Central, #01-792, Singapore 440080, Singapore.

E-mail addresses: andrew.wee@singhealth.com.sg, aweekh@singnet.com.sg.

<https://doi.org/10.1016/j.mehy.2020.110374>

Received 14 June 2020; Received in revised form 20 August 2020; Accepted 22 October 2020

Available online 12 November 2020

0306-9877/© 2020 The Author(s).

Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

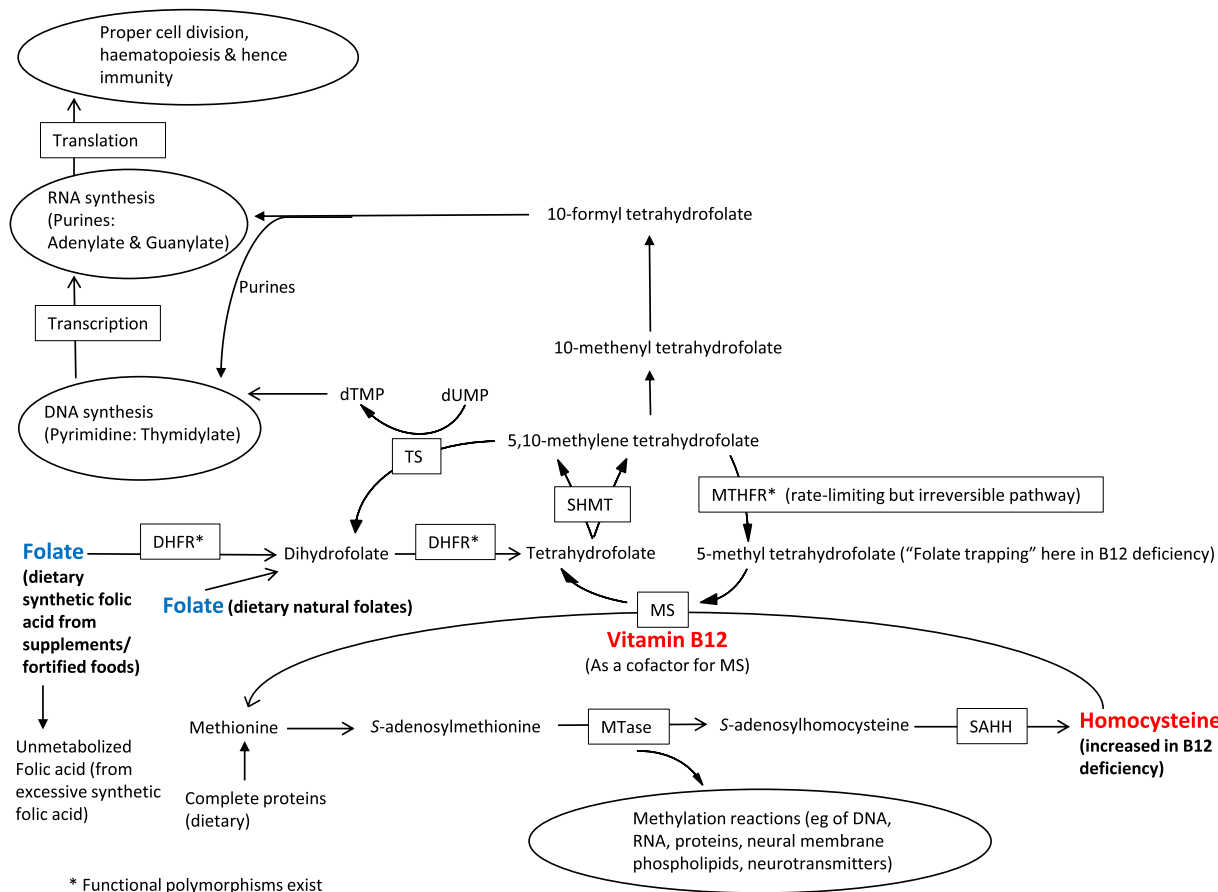


Fig. 1. One-carbon metabolism – an overview of the functional relationships between vitamin B12, folate, and homocysteine towards DNA syntheses, cellular regulation, and body repair. Its components need to be balanced for this system to be optimally tuned. Dietary factors and functional genetic polymorphisms of its enzymes exist to affect this balance. DHFR, Dihydrofolate reductase; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; MS, methionine synthase; MTase, methyltransferase; MTHFR, methylenetetrahydrofolate reductase; SAHH, S-adenosylhomocysteine hydrolase; SHMT, serine hydroxymethyltransferase; TS, thymidylate synthase.

to 29% [5].

Without restriction to old age or diabetes, globally reported CFRs are lower. As of May 2020, the highest case fatality rate of 9.6% was in Europe, followed by 5.9% in North America, and 3.5% in Asia [15]. The Diamond Princess cruise ship, where 100% of the population (mean age of 58 years) was tested, had a corrected infection fatality rate of 1.3% [16]. In contrast, the CFR of severe seasonal flu is only 0.1% [17].

In particular, among the nations with more than 1000 documented cases of COVID-19, Singapore reports the lowest CFR of only 0.07% [18]. As of 01 June 2020, with its extensive contact tracing and testing of suspected cases, Singapore had up to 35,292 cumulative cases of COVID-19 infections, but with only 7 patients receiving intensive care at that time and an official case fatality of only 23 individuals, with men outnumbering women (16 men (70%) vs. 7 (30%) women) [19]. Excluding the youngest to die (a 58-year-old woman), all who died were aged ≥60 years with the mean age (±SD) for men significantly younger than that for women (74.4 (±10.1) years vs. 86.7 (±7.0) years respectively) [dataset] [20] (Author’s analysis: *P* = 0.02 using the two-tailed *t*-test; STATA 13.0). Notably, 33,027 (94%) of the infected comprised relatively young blue-collar non-citizen workers residing in dormitories. Predominantly from South-, East-, and Southeast Asia, many of these workers had, in contrast, mild or no symptoms and none had died from COVID-19 at the time of writing [19].

Established reasons for the higher COVID-19 complications in the elderly and those with diabetes include glycaemic instability, impaired T-cell response, and pre-existing comorbidities (like obesity, cardiovascular and kidney diseases) [1,21]. Postulated reasons include chronic

low-grade inflammation (that is associated with increased cytokines) and factors associated with viral entry (like ACE-2 expression) [1,4,21,22].

Apart from old age and diabetes, other prognostic markers for poor COVID-19 outcomes include: **higher** BMI [5,23], ALT [24], CRP [5], CD4+:CD8+ ratios [25], pro-inflammatory cytokine levels (eg IL-2 and TNF-α) [4,24,25], D-dimer levels [24,26], and IFN-γ levels [24]; **lower** eGFR [5], peripheral counts of CD4+ and CD8+ T cells [25], and lymphocyte count [5]; and the male gender [7]. However, all these risk factors are either non-modifiable or merely reflect irreversibly late sequelae.

Recently, it has been proposed that nutrition is partly responsible for these wide differences in COVID-19 death rates seen between (and even within) countries [27]. Nutrition can certainly affect the immune system’s ability to protect against viral infections [28,29]; elderly malnutrition in COVID-19 infections has been described in a Chinese study. This study of 182 elderly (age ≥65 years) COVID-19 patients using the Mini-Nutritional-Assessment score showed 52.7% to be “malnourished” and a further 27.5% to be “at risk of malnutrition”. Regression risk in the study also showed diabetes mellitus to be an independent risk factor for malnutrition (OR: 2.12; 95% CI: 1.92–3.21; *P* = 0.006) [30].

There are now more than 40 registered studies looking at the role of vitamins in COVID-19 [31]. However, most are focused on vitamins C, D and the mineral zinc—and vitamin B12 deficiency’s impact on COVID-19 are currently unclear.

Hypothesis

We therefore sought to review the hypothesis that vitamin B12 deficiency, along with its resultant defects in one-carbon metabolism—a central group of vitamin B12-folate-homocysteine dependent biochemical reactions that are crucial for wide-ranging aspects of DNA syntheses, cellular regulation and body repair [32,33]—could be a major modifiable risk factor in COVID-19 morbidity and mortality in the elderly and those with diabetes mellitus.

Evaluation of the hypothesis

Search strategy and selection criteria

To review the state of the art pertaining to vitamin B12 deficiency (henceforth known as “B12” deficiency) and COVID-19, an on-line literature search on “Pubmed”, “Google”, and the clinical trials register “ClinicalTrials.gov” was done using the following MeSH and free text search terms (associated with B12 deficiency): “B12 deficiency”; “B12”; “folate”; “vitamin”; “homocysteine”; “one-carbon metabolism”; and “metformin”, which were first used singly, and subsequently matched, each in turn, with the terms (associated with COVID-19): “COVID”; “COVID-19”; and “Sars-Cov-2”. A third round of searches ensued with the terms (comprising biomarkers of poor COVID-19 outcomes, or the means by which the virus gains entry): “prognostic factors”; “elderly”; “older”; “diabetes”; “obesity”; “BMI”; “gender”; “tnf alfa”; “interleukin”; “ifn gamma”; “cd4 cd8”; “lymphocyte”; “nk cell”; and “ace” added in turn to the first round of searches. Only published articles in English were shortlisted and, where appropriate, filters were used to extract only reviews. In the event of similar reviews, the most recent or most comprehensive was chosen. Reference lists from journal articles and book chapters resulting from the online-search, together with the author’s past reading on the topic, were also used. Given the unprecedented rapid pace of COVID-19 research, preprints were judiciously allowed in the search and qualified as such in citations.

B12 deficiency is associated with the elderly and those with diabetes

Old age per se associates with B12 deficiency; deficiencies have been reported in up to 15% of elderly populations in the USA and Europe [34–37]. This has been attributed to B12 malabsorption (like pernicious anaemia and atrophic gastritis) [34–37], to poorer nutrition or diets weighted on vegetables [38], or to increased urinary and gut loss of B12 [39] through age-related defects in the megalin-cubulin-amnionless complex of receptors (that are responsible for reclaiming transcobalamin-bound B12 from the glomerular filtrate and intrinsic-factor-bound B12 in the gut) [40–42].

Diabetes further associates with B12 deficiencies worldwide. A U.S. primary-care services study [43] showed that 22% of outpatients had metabolically confirmed vitamin B12 deficiency. Similarly, the prevalence of vitamin B12 deficiency was 28.1% in a cross-sectional study of 550 type-2 diabetes patients in four primary care centres in the Netherlands [44]. Our polyclinic’s pilot cross-sectional study of 56 elderly patients with type-2 diabetes showed that 43% were vitamin B12 deficient, of whom 75% had concurrent hyperhomocysteinaemia [45].

Several reasons for B12 deficiency in T2DM have been proposed. The drug “metformin” is the mainstay of type-2-diabetes treatment [46,47] and there is consistently strong evidence of its association with B12 deficiency [48–51]. Metformin reportedly disrupts calcium-dependent intestinal absorption of vitamin B12 and calcium supplementation was shown to reverse this [52]. The frequent use of proton-pump inhibitors or histamine H2 receptor antagonists among these patients [38,53], especially as gastric-acid-reducing adjuncts to aspirin therapy (used in the secondary prevention of cardiovascular events in diabetes [54,55]), could also reduce the ability to digest B12 from food [56].

B12 deficiency and one-carbon metabolism

Vitamin B12 has critical functions (through one-carbon metabolism) (Fig. 1) in both cellular and humoral immunity [29,57]. B12 is a cofactor for methionine synthase and B12 deficiency results in the wasted accumulation (“trapping”) of 5-methyl tetrahydrofolate that is formed by the rate-limiting but irreversible reaction of the enzyme “methyl-ene tetrahydrofolate reductase” (MTHFR) [57]. This could result in a “secondary folate deficiency” with impairments in thymidine and purine synthesis, ultimately impacting DNA and RNA synthesis that are vital for proper cell division, haematopoiesis and hence immunity [29,57,58].

B12 deficiency and the immune system

A Japanese study [59] on 11 B12-deficient patients and 13 non-deficient controls demonstrated that B12-deficient patients had statistically higher CD4+:CD8+ ratios ($P < 0.05$); lower NK cell activity ($P < 0.01$); reduced CD8+ cell (cytotoxic T cell) count ($P < 0.01$); and lymphopaenia ($P < 0.01$) (all markers implying lowered immunity). After only a 2-week administration of B12 (using methylcobalamin 500 µg/day) in both patients and 8 of the initial 13 controls, B12-deficient patients demonstrated significant improvements in leucopaenia ($P < 0.05$); lymphopaenia ($P < 0.05$); CD8+ cell count ($P < 0.05$); and NK cell activity ($P < 0.01$). Even healthy controls had a better immune profile after B12 administration as evidenced by increased lymphocyte and CD8+ cell counts (both, $P < 0.05$)—suggesting anti-viral immunological benefits at B12 levels beyond normally accepted ranges. The authors posited that B12 prevents the apoptosis of lymphocytes, especially the CD8+ cells [59].

A later prospective Turkish study on 30 B12-deficient patients with pernicious anaemia also showed restoration in lymphocyte counts, NK cell activity and CD4+:CD8+ ratios after intramuscular administration of cyanocobalamin [60]. In addition, this study demonstrated increase in the levels of additional anti-viral components of the immune system, namely: complements C3 and C4, and immunoglobulins.

B12 deficiency and the microbiome in the gut

Like us, human gut microbes that support our gut barrier also require B12 as a cofactor for metabolism [29]. Moreover, rather than contribute to our B12 levels, these human microbes have multiple corrinoid (B12-like)-dependent enzymatic functions that make them likely competitors for our dietary cobalamin; a shortage of which potentially affects the proper balance of gut microbial communities (termed “gut dysbiosis”) and ultimately our health. Elderly people have lower gut microbiome diversity and beneficial microbes like Bifidobacterium [61]. A senescent gut thus lends itself to pathological bacteria (pathobiont) overgrowth, increased gut permeability to bacteria and toxins, and ultimately increase proinflammatory cytokines [61]. In addition, administration of Bifidobacterium lactis into 30 healthy elderly volunteers showed improved anti-viral NK-cell activity [62]. There is also evidence of a bidirectional “gut-lung axis” whereby microbiomes between lung and gut affect each other [63], in keeping with the respiratory and gastrointestinal clinical manifestations of COVID-19. Indeed, alterations in gut microbiota correlating with disease severity have been described in 15 patients with COVID-19 [64].

B12 deficiency and obesity

Obesity, a prevalent non-modifiable risk factor for COVID-19 complications [5], associates inversely with both B12 [65] and folate levels [66] and directly with homocysteine levels [67]. A nationally representative sample of 9,075 U.S. adults showed significantly higher odds of obesity (defined as BMI ≥ 30 kg/m²) with lower quartiles of B12 levels (P for trend < 0.001) [65]; whilst a study on 1462 pregnant Korean women showed consistent negative correlation between BMI

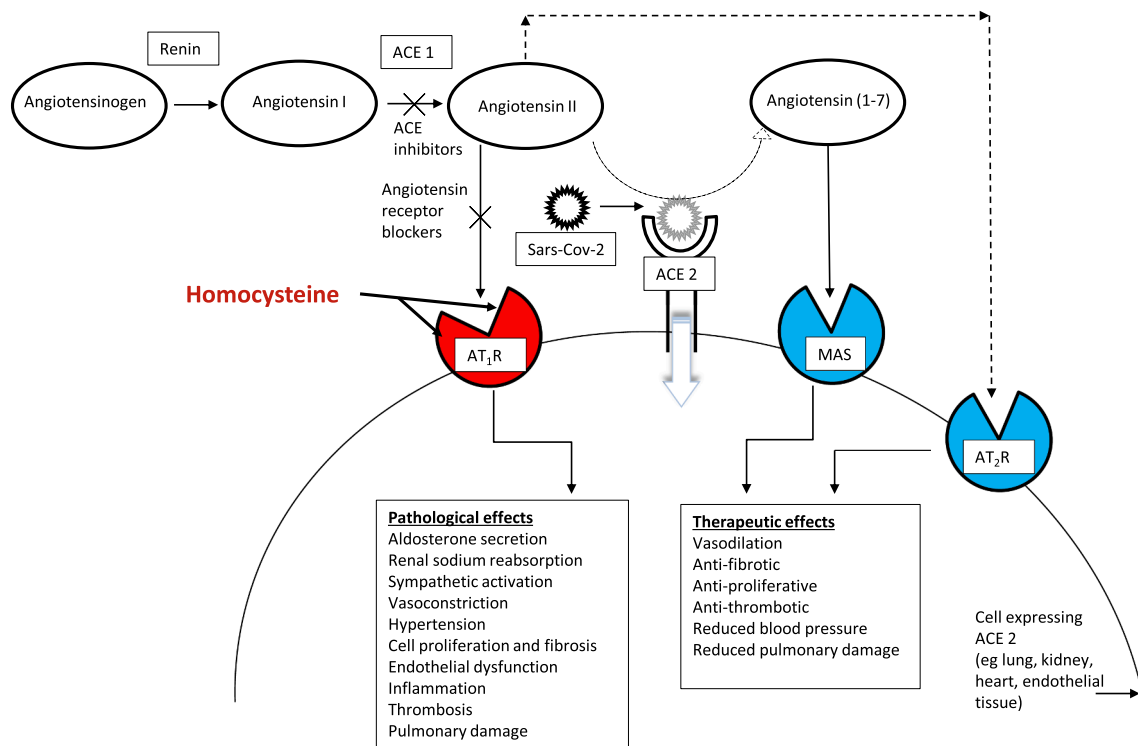


Fig. 2. Homocysteine and the Sars-Cov-2 virus in relation to the renin-angiotensin-aldosterone system. Sars-Cov-2 gains entry via ACE-2 while homocysteine binds orthosterically and allosterically to AT₁R, activating it (and displacing Angiotensin II). AT₁R, angiotensin II type 1 receptor; AT₂R, angiotensin II type 2 receptor; ACE inhibitors, angiotensin-converting enzyme inhibitors; ACE-1, angiotensin-converting enzyme 1; ACE-2, angiotensin-converting enzyme 2; MAS, Mas (mitochondrial assembly 1) G protein-coupled receptor.

and serum folate in mid- and late pregnancy ($P = 0.001$ and 0.024 , respectively). A Danish study on 118 patients in 2018 showed a positive correlation of BMI with homocysteine ($P = 0.005$) [67]. With regard to visceral obesity, a Turkish case-control study (comprising 45 patients with non-alcoholic fatty liver disease vs. 30 healthy controls) showed a significant negative correlation of B12 levels with both ALT levels and the grade of liver disease, as well as significantly lower B12 levels in the cases compared with controls ($P < 0.05$) [68].

B12 deficiency extends its reach through homocysteine

Homocysteine is a metabolic consequence of B12 deficiency (and also folate imbalance as discussed below). Renally cleared [69], it has been linked to adverse cardiovascular and neurocognitive outcomes [70]. Like adverse COVID-19 complications, homocysteine has also been associated with the male gender [70,71] and higher D-dimer levels (a marker of thrombosis and coagulopathy seen in COVID-19) [13,72].

Homocysteine has been shown to be a pro-inflammatory amino acid that works identical to (but yet independent of) IFN- γ in its ability to induce inflammatory transcriptional signalling in monocytes [73]. The authors found that both homocysteine and IFN- γ each induced a separate and distinct set of cytokine genes (those genes induced by homocysteine include IL6, IL8 and TNF α). These genes in turn functionally converge and bind very strongly (through their promoters) to a set of 5 transcriptional factors (namely: heat shock factor; monocyte enhancer factor-2; nuclear factor of activated T-cells; nuclear factor kappa light chain enhancer of activated B cells; krueppel-like factor 4)—hence plausibly leading to duplicate and synergistic inflammatory signal cascades between homocysteine and IFN- γ ; ultimately contributing to monocyte-mediated vascular inflammation [13,73].

Biomolecularly, homocysteine has also been shown to bind strongly to the angiotensin 1 receptor (AT₁R) and activating its deleterious downstream signalling effects. It binds the receptor both orthosterically

and allosterically [22,74], and with such affinity as to be able to displace angiotensin II from AT₁R (Fig. 2).

Indeed clinically, it was recently published that hyper-homocysteinaemia (defined in the paper as greater than $15.5 \mu\text{mol/L}$) was significantly associated with adverse pulmonary progression on CT imaging in 273 COVID-19 patients ($P < 0.05$ for both univariate and logistic regression analyses) [75], lending weight to its role in COVID-19 morbidity.

It was also recently described that the SARS-Cov-2 virus has a non-structural protein (nsp14) that is in fact a viral methyltransferase (specifically: guanidine-N7-methyltransferase) [76,77]. This enzyme likely uses the host's S-adenosylmethionine for methylation of mRNA caps (a process believed to be essential for viral replication), potentially generating homocysteine over and above that from our own human methyltransferase (and perhaps leaving less for say methylation of neural myelin)—especially in the presence of B12 deficiency [58,76,77] (Fig. 1).

B12 as an adjunct to antiviral therapy

As an RNA virus, a significant portion of the Sars-Cov-2 genome codes for a main polypeptide protease (M^{pro}), that is a key enzyme for the virus' replication and thus an attractive target for potential anti-viral drugs [78]. Computer-aided molecular screening of the full array of FDA-approved drugs (with the aim of repurposing for the use against M^{pro}), ranked vitamin B12 in the 4th position and a potentially safe drug to be repurposed for COVID-19 therapy [79]. Benchmarked against curcumin (a well-known [79] and stronger inhibitor of M^{pro} than hydroxychloroquine [80]), vitamin B12 had a relative docking affinity for M^{pro} that is 1.99 times better than curcumin (even rivalling ribavirin's 2nd-placed affinity of 2.01 times). A separate computational study, a preprint nevertheless, reports that B12 potentially inhibits another aspect of the Sars-Cov-2 virus: that of its non-structural protein12 (an

RNA-dependent-RNA polymerase that is also vital for the virus' replication) [81].

The concept of B12 as an adjuvant or adjunct to antiviral therapy is not new, for a precedent exists in its activity against the Hepatitis C virus (HCV)—itself also a mutation-prone positive-sense single-stranded RNA virus, against which no vaccine yet exists [82]. HCV requires an internal ribosomal entry site (IRES) to mediate the initiation of its translation (and hence its replication process); B12 has been shown to inhibit IRES [83]. Based on this finding, two randomized controlled trials—an Italian followed by an Iranian [84,85]—reported the vitamin's ability to improve rates of sustained viral response (SVR) when added to pegylated interferon- α -plus-ribavirin combined therapy, the erstwhile standard of care (SOC) for hepatitis C infections (from 2001 to 2011) [85]. On its own, SOC yields a less-than-perfect SVR of only 40–50% in the most-difficult-to-treat of the HCV genotypes (i.e. genotype 1) [84].

In the Italian study [84] on 94 patients (divided equally between intervention (Vitamin B12 + SOC) and control groups (SOC only)), SVR was achieved in 72% receiving intervention compared to only 38% receiving control; a 34% improvement in SVR ($P = 0.001$). Furthermore, this improvement in SVR through intervention was more pronounced in patients who also had the refractory HCV genotype 1 (41%, $P = 0.003$) or the highest baseline viral load (38%, $P = 0.001$).

In the Iranian trial [85] on 36 patients receiving intervention (B12 + SOC) vs. 38 controls (SOC only), SVR was achieved in 80% of patients on intervention compared to only 68% of those on control ($P = 0.0001$). This study also demonstrated significantly reduced treatment side effects and dropout rates in the intervention arm ($P = 0.0001$).

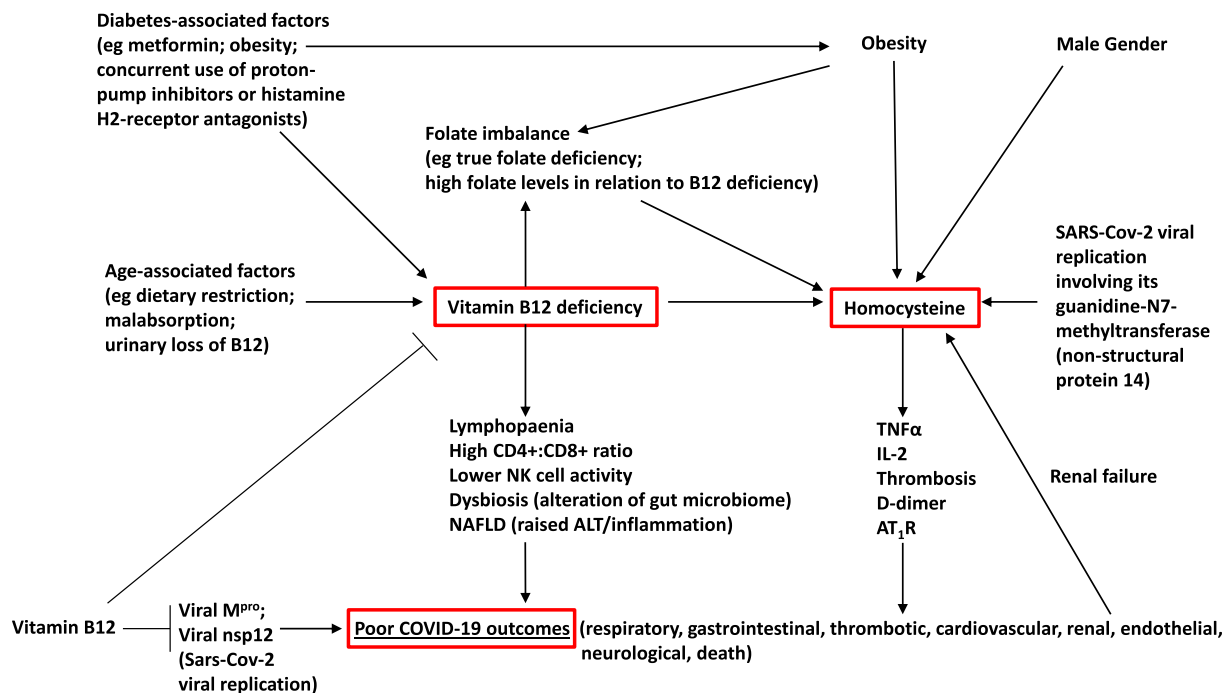
Nevertheless, pegylated interferon and ribavirin have been supplanted by direct-acting antivirals which yield superior SVRs—and hence the use of B12 as an adjunct to treating HCV infection in clinical practice was a lost opportunity [86,87].

B12 and COVID-19 (emerging studies)

Currently, 2 other emerging studies point to defective one-carbon metabolism and poor COVID-19 outcomes. The first study (a preprint) comprised 43 COVID-19 patients aged ≥ 50 years, 17 of whom received a combination of vitamin D, magnesium and vitamin B12. It showed significantly lower odds of needing “oxygen therapy” and/or “intensive care support” (OR: 0.152; 95% CI: 0.025–0.930; $P = 0.041$) [88]. The second, an Israeli cohort of 162 patients, showed that lower folate levels consistently associated with increasing stages of COVID-19 severity ($P = 0.005$). Interestingly, higher levels of vitamin B12 was associated with increased severity of COVID-19 ($P = 0.039$) [89]. However, this paper did not publish multivariable analyses, nor did the authors explore statistically the potential interaction between folate and B12 [89].

B12 deficiency and the need for one-carbon metabolism to be balanced and fine-tuned

Nevertheless, the findings in the Israeli publication above highlights the clinical importance of an optimal balance between B12 and folate. Folate supplementation in the presence of B12 deficiency is known to both mask the haematological, but yet potentially exacerbate the neurological aspects of B12 deficiency [58,90]. Excessive folate intake from synthetic folic acid supplements (or food fortification) manifests as unmetabolized folic acid (UMFA). UMFA was detected in one-third of elderly participants in the 1999–2002 U.S. NHANES and, in the presence of low B12 levels, UMFA was associated with lower cognitive test scores and lower mean red blood cell volume in seniors [90]. Moreover, from this NHANES data, it was observed that in the presence of B12 deficiency, higher homocysteine levels (and methylmalonate levels) occurred when folate levels were high [90,91]. Another American study on 105 healthy postmenopausal women revealed that 78% of them had detectable plasma UMFA, and that these women with detectable UMFA had 23% lower NK cell cytotoxicity compared with those without ($P =$



Direction of arrows denote published associations discussed in the text; not necessarily causality.

Fig. 3. Pictorial summary of vitamin B12 deficiency's association with biomarkers of adverse COVID-19 outcomes (refer to text for details). AT₁R, angiotensin II type 1 receptor; NAFLD, non-alcoholic fatty liver disease; M^{pro}, main polypeptide protease of Sars-Cov-2; nsp12, non-structural protein12 of Sars-Cov-2 (an RNA-dependent-RNA polymerase).

0.04) [90,92]. Such a scenario could plausibly occur in countries where there is mandatory folate fortification of food (eg The Americas, Australia, and Indonesia) relative to variable (and possibly unreliable) dietary B12 consumption [90].

Why B12 deficiency still exists

Vitamin B12 deficiency poses an already-existing risk in older patients and those with diabetes mellitus through possible adverse neurocognitive and haematological consequences [38,53]. Currently, the American [46] and Singaporean [93] 2017 guidelines on the treatment of type-2 diabetes mellitus with metformin recommend the periodic measurement of vitamin B12 levels and their supplementation if necessary, particularly in patients with “anaemia or peripheral neuropathy”. However, these are insidious (hence difficult to recognize), late, and sadly often irreversible sequelae of B12 deficiency—and the guidelines fall short of the annual screening that was proposed almost 50 years ago [51]. Indeed, regular screening for metformin-associated B12 deficiency in diabetes is still wanting; a 2019 Irish audit of 241 patient records (within both primary and specialist care) found that 56 (23%) patients were never screened for B12 levels [94].

Consequences of the hypothesis and discussion

Vitamin B12 deficiency is a prevalent disorder that should be addressed and prevented in its own right. In view of this hypothesis and the COVID-19 crisis, we recommend that it should be more urgently addressed; the elderly and those with diabetes mellitus should be regularly screened for deficiencies, whereupon B12 levels should be optimized and balanced carefully in tandem with folate (perhaps using homocysteine as a gauge that one-carbon-metabolism has been optimally tuned).

Urgent research is needed to explore B12 deficiency’s role as a possible confounder in COVID-19’s devastating toll on the elderly and those with diabetes. As such, current research and clinical trials could add the testing of vitamin B12, folate and homocysteine levels into their protocols. Where possible, holotranscobalamin and methylmalonic acid levels could be included as better/supplementary biomarkers of B12 deficiency because diagnosing deficiency using B12 levels alone has problems with sensitivity and specificity [84,95–97]. For example, peripheral/serum B12 levels could be falsely elevated in liver disease [84] and myeloproliferative diseases [95]—despite true metabolic B12 deficiencies—leading to underdiagnoses of deficiencies [95].

If the hypothesis is indeed true, further research should also factor in and adjust for functional genetic polymorphisms in one-carbon metabolism (eg DHFR19bpdel [90] and MTHFR677C → T [90]) as well as the use of metformin (given emerging evidence of metformin’s association with better COVID outcomes [5,98,99] that is seemingly at odds with its association with B12 deficiency [48–50]). Going beyond deficiency in B12, further research could also include finding the optimal therapeutic levels of B12 (vis-à-vis folate and homocysteine levels)—either as an adjuvant to mainstream treatments or by itself—against COVID-19.

Summary and conclusion

This review shows that B12 deficiency associates in multiple areas very similar to where COVID-19 exerts its damaging effects: immunologically (innate and adaptive; cellular and humoral responses; and inflammation); microbiologically (gut microbiome); haematologically (coagulation); and endothelial cell signalling (more inflammation). These associations have the potential to converge, synergise and finally add to the disproportionate toll COVID-19 inflicts on the elderly and those with diabetes. There is also emerging data that B12 per se could interfere with Sars-Cov-2 replication, adding a potential therapeutic dimension. B12 deficiency is thus a possibly modifiable (and certainly avoidable) risk factor in our fight against COVID-19 (Fig. 3).

Useful website

National Institute of Health-Office of Dietary Supplements. Vitamin B12, Fact Sheet for Health Professionals. Available at: <https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/>.

Disclaimer

The opinions expressed by the author of this manuscript does not necessarily reflect the official position of the institutions with which the author is affiliated to, or the funder.

Open researcher and contributor identification numbers

0000000236801614 Andrew Kien Han Wee.

Declaration of Competing Interest

The author declares no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

Acknowledgement

I extend my deepest gratitude to Ms. Fion Farn of Duke-NUS Centre for Clinician-Scientist Development (CCSD) for her administrative support.

Sources of support

1. ExxonMobil-NUS Research Fellowship for Clinicians, 2014.
2. National Medical Research Council Clinician Investigator Salary Support Programme. Reference Number: NMRC/CISSP/2014/030.
3. Duke-NUS Centre for Clinician-Scientist Development (CCSD) Khoo Scholars Program 2018.

Ethics approval

Not applicable.

Author’s contribution

Andrew Kien Han Wee is responsible for all aspects of this manuscript.

References

- [1] Shahid Z, Kalayanamitra R, McClafferty B, et al. COVID-19 and older adults: what we know. *J Am Geriatr Soc* 2020;68:926–9.
- [2] Huang I, Lim MA, Pranata R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia – a systematic review, meta-analysis, and meta-regression. *Diabetes Metab Syndr* 2020;14:395–403.
- [3] Kumar A, Arora A, Sharma P, et al. Is diabetes mellitus associated with mortality and severity of COVID-19? a meta-analysis. *Diabetes Metab Syndr* 2020;14:535–45.
- [4] Angelidi AM, Belanger MJ, Mantzoros CS. COVID-19 and diabetes mellitus: What we know, how our patients should be treated now, and what should happen next. *Metabolism* 2020:154245.
- [5] Cariou B, Hadjadj S, Wargny M, et al. Phenotypic characteristics and prognosis of inpatients with COVID-19 and diabetes: the CORONADO study. *Diabetologia* 2020.
- [6] Desai R, Singh S, Parekh T, Sachdeva S, Sachdeva R, Kumar G. COVID-19 and diabetes mellitus: a need for prudence in elderly patients from a pooled analysis. *Diab Metab Syndrome: Clin Res Rev* 2020;14:683–5.
- [7] Li X, Xu S, Yu M, Wang Ke, Tao Yu, Zhou Y, Shi J, Zhou M, Wu Bo, Yang Z, Zhang C, Yue J, Zhang Z, Renz H, Liu X, Xie J, Xie M, Zhao J. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020; 146(1):110–8. <https://doi.org/10.1016/j.jaci.2020.04.006>.
- [8] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang Li, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019

- pneumonia in Wuhan, China. *JAMA Intern Med* 2020;180(7):934. <https://doi.org/10.1001/jamainternmed.2020.0994>.
- [9] United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Ageing 2019: Highlights (ST/ESA/SER.A/430). Internet: https://www.google.com.sg/url?sa=t&rc=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwJl_uGqPLpAhXBSH0KHdNgBcQQFJAegQIBB&url=https%3A%2F%2Fwww.un.org%2Fen%2Fdevelopment%2Fdesa%2Fpopulation%2Fpublications%2Fpdf%2Fageing%2FWorldPopulationAgeing2019-Highlights.pdf&usg=AOvVaw0C2HroVeVv_ijqLZBxbVhS. Accessed on 08 June 2020.
- [10] World Health Organization. Diabetes. Internet: https://www.who.int/health-topics/diabetes#tab=tab_2. Accessed 08 June 2020.
- [11] Lippi G, Sanchis-Gomar F, Henry BM. Coronavirus disease 2019 (COVID-19): the portrait of a perfect storm. *Ann Transl Med* 2020;8:497.
- [12] Baek WK, Sohn SY, Mahgoub A, Hage R. A comprehensive review of severe acute respiratory syndrome coronavirus 2. *Cureus* 2020;12:e7943.
- [13] Sardu C, Gambardella J, Morelli MB, Wang X, Marfella R, Santulli G. Hypertension, thrombosis, kidney failure, and diabetes: is COVID-19 an endothelial disease? a comprehensive evaluation of clinical and basic evidence. *J Clin Med* 2020;9.
- [14] Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395:1033–4.
- [15] Dongarwar D, Salihu HM. COVID-19 Pandemic: marked global disparities in fatalities according to geographic location and universal health care. *Int J MCH and AIDS* 2020;9:213–6.
- [16] Russell TW, Hellewell J, Jarvis CI, et al. Estimating the infection and case fatality ratio for coronavirus disease (COVID-19) using age-adjusted data from the outbreak on the Diamond Princess cruise ship, February 2020. *Eurosurveillance* 2020;25:2000256.
- [17] Fauci AS, Lane HC, Redfield RR. Covid-19 — navigating the uncharted. *N Engl J Med* 2020;382:1268–9.
- [18] Statista. Coronavirus (COVID-19) death rate in countries with confirmed deaths and over 1,000 reported cases as of June 3, 2020, by country. Internet: <https://www.statista.com/statistics/1105914/coronavirus-death-rates-worldwide/>. Accessed on 01 June 2020.
- [19] Ministry of Health SINGAPORE. Updates on COVID-19 (Coronavirus Disease 2019) Local Situation. Internet: <https://www.moh.gov.sg/covid-19/situation-report>. Accessed on 01 June 2020., (2020).
- [20] A. Wee, Singapore Covid-19 Deaths up to 01 June 2020 by Age and Gender (dataset collated from local news). Internet: <https://doi.org/10.13140/RG.2.2.11213.67041>. (2020).
- [21] Gupta R, Hussain A, Misra A. Diabetes and COVID-19: evidence, current status and unanswered research questions. *Eur J Clin Nutr* 2020;1–7.
- [22] Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med* 2020;76:14–20.
- [23] Tamara A, Tahapary DL. Obesity as a predictor for a poor prognosis of COVID-19: a systematic review. *Diab Metab Syndrome: Clin Res Rev* 2020;14:655–9.
- [24] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2019;395(2020):497–506.
- [25] Muniyappa R, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. *Am J Physiol Endocrinol Metab* 2020;318:E736–e741.
- [26] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054–62.
- [27] Bousquet J, Anto JM, Iaccarino G, et al. Is diet partly responsible for differences in COVID-19 death rates between and within countries? *Clin Transl Allergy* 2020;10:16.
- [28] Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients* 2020;12.
- [29] Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune system-working in harmony to reduce the risk of infection. *Nutrients* 2020;12.
- [30] Li T, Zhang Y, Gong C, et al. Prevalence of malnutrition and analysis of related factors in elderly patients with COVID-19 in Wuhan, China. *Eur J Clin Nutr* 2020;74(6):871–5. <https://doi.org/10.1038/s41430-020-0642-3>.
- [31] NIH. U.S. National Library of Medicine. ClinicalTrials.gov. Internet: <https://clinicaltrials.gov/ct2/results?cond=COVID&term=vitamin&cntry=&state=&city=&dist=>. Accessed on 02 June 2020.
- [32] Selhub J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *J Nutr Health Aging* 2002;6:39–42.
- [33] Suh E, Choi S-W, Friso S. Chapter 36 – one-carbon metabolism: an unsung hero for healthy aging. In: Malavolta M, Mocchegiani E, editors. *Molecular basis of nutrition and aging*. San Diego: Academic Press; 2016. p. 513–22.
- [34] Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. *Annu Rev Nutr* 2004;24:299–326.
- [35] Clarke R, Grimley Evans J, Schneede J, et al. Vitamin B12 and folate deficiency in later life. *Age Ageing* 2003;33:34–41.
- [36] Laird EJ, O'Halloran AM, Carey D, O'Connor D, Kenny RA, Molloy AM. Voluntary fortification is ineffective to maintain the vitamin B12 and folate status of older Irish adults: evidence from the Irish Longitudinal Study on Ageing (TILDA). *Br J Nutr* 2018;120:111–20.
- [37] Green R, Allen LH, Bjorke-Monsen AL, et al. Vitamin B12 deficiency. *Nat Rev Dis Primers* 2017;3:17040.
- [38] Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. *BMJ* 2014;349:g5226.
- [39] Pannerec A, Migliavacca E, De Castro A, et al. Vitamin B12 deficiency and impaired expression of amnionless during aging. *J Cachexia, Sarcopenia Muscle* 2018;9:41–52.
- [40] Christensen EI, Birn H. Megalin and cubilin: multifunctional endocytic receptors. *Nat Rev Mol Cell Biol* 2002;3:256–66.
- [41] Moestrup SK, Kozyraki R, Kristiansen M, et al. The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral membrane protein with homology to developmental proteins. *J Biol Chem* 1998;273:5235–42.
- [42] De S, Kuwahara S, Saito A. The endocytic receptor megalin and its associated proteins in proximal tubule epithelial cells. *Membranes (Basel)* 2014;4:333–55.
- [43] Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. *J Am Board Fam Med* 2009;22:528–34.
- [44] Beulens JW, Hart HE, Kuijs R, Kooijman-Buiting AM, Rutten GE. Influence of duration and dose of metformin on cobalamin deficiency in type 2 diabetes patients using metformin. *Acta Diabetol* 2015;52:47–53.
- [45] Wee AK. Serum folate predicts muscle strength: a pilot cross-sectional study of the association between serum vitamin levels and muscle strength and gait measures in patients >65 years old with diabetes mellitus in a primary care setting. *Nutr J* 2016;15:89.
- [46] Chamberlain JJ, Herman WH, Leal S, et al. Pharmacologic therapy for type 2 diabetes: synopsis of the 2017 American Diabetes Association Standards of Medical Care in Diabetes. *Ann Intern Med* 2017;166:572–8.
- [47] Internal Clinical Guidelines Team. Type 2 Diabetes in Adults: Management. London: National Institute for Health and Care Excellence (UK); 2015 Dec. (NICE Guideline, No. 28.) Internet: <https://www.ncbi.nlm.nih.gov/books/NBK338142/>. Accessed on 24 July 2018.
- [48] de Jager J, Kooy A, Leher P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ* 2010;340:c2181.
- [49] Aroda VR, Edelstein SL, Goldberg RB, et al. Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. *J Clin Endocrinol Metab* 2016;101:1754–61.
- [50] Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. *PLoS ONE* 2014;9:e100379.
- [51] Tomkin GH, Hadden DR, Weaver JA, Montgomery DA. Vitamin-B12 status of patients on long-term metformin therapy. *Br Med J* 1971;2:685–7.
- [52] Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care* 2000;23:1227–31.
- [53] Stabler SP. Clinical practice. Vitamin B12 deficiency. *N Engl J Med* 2013;368:149–60.
- [54] Vonkeman H, Meek I, van de Laar M. Risk management of risk management: Combining proton pump inhibitors with low-dose aspirin. *Drug, Healthcare Patient Safety* 2010;2:191–204.
- [55] Chamberlain JJ, Rhinehart AS, Shaefer Jr CF, Neuman A. Diagnosis and management of diabetes: Synopsis of the 2016 American diabetes association standards of medical care in diabetes. *Ann Intern Med* 2016;164(2016):542–52.
- [56] Lam JR, Schneider JL, Zhao W, Corley DA. Proton pump inhibitor and histamine 2 receptor antagonist use and vitamin B12 deficiency. *JAMA* 2013;310:2435–42.
- [57] Saeed F, Nadeem M, Ahmed RS, Tahir Nadeem M, Arshad MS, Ullah A. Studying the impact of nutritional immunology underlying the modulation of immune responses by nutritional compounds – a review. *Food Agric Immunol* 2016;27:205–29.
- [58] Scott JM, Weir DG. The methyl folate trap. A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid induced exacerbation of subacute combined degeneration in pernicious anaemia. *Lancet* 1981;2:337–40.
- [59] Tamura J, Kubota K, Murakami H, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol* 1999;116:28–32.
- [60] Erkurt MA, Aydogdu I, Dikilitaş M, et al. Effects of cyanocobalamin on immunity in patients with pernicious anemia. *Med Princ Pract* 2008;17:131–5.
- [61] Nagpal R, Mainali R, Ahmadi S, et al. Gut microbiome and aging: Physiological and mechanistic insights. *Nutr Healthy Aging* 2018;4:267–85.
- [62] Gill HS, Rutherford KJ, Cross ML, Gopal PK. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. *Am J Clin Nutr* 2001;74:833–9.
- [63] Dhar D, Mohanty A. Gut microbiota and Covid-19- possible link and implications. *Virus Res* 2020;285:198018.
- [64] Zuo T, Zhang F, Lui GCY. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology* 2020;159(3):944–955.e8. <https://doi.org/10.1053/j.gastro.2020.05.048>.
- [65] Sun Y, Sun M, Liu B, et al. Inverse association between serum vitamin B12 concentration and obesity among adults in the United States. *Front Endocrinol (Lausanne)* 2019;10.
- [66] Kim H, Hwang JY, Kim KN, et al. Relationship between body-mass index and serum folate concentrations in pregnant women. *Eur J Clin Nutr* 2012;66:136–8.
- [67] Lind MV, Lauritzen L, Vestergaard H, et al. One-carbon metabolism markers are associated with cardiometabolic risk factors. *Nutr Metab Cardiovasc Dis* 2018;28:402–10.
- [68] Koplay M, Gulcan E, Ozkan F. Association between serum vitamin B12 levels and the degree of steatosis in patients with nonalcoholic fatty liver disease. *J Investig Med* 2011;59:1137–40.

- [69] Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. *J Am Soc Nephrol* 2001;12:2181.
- [70] Refsum H, Nurk E, Smith AD, et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* 2006;136:1731s–40s.
- [71] Cohen E, Margalit I, Shochat T, Goldberg E, Krause I. Gender differences in homocysteine concentrations, a population-based cross-sectional study. *Nutr Metab Cardiovasc Dis* 2019;29:9–14.
- [72] Kuch B, Bobak M, Fobker M, et al. Associations between homocysteine and coagulation factors—a cross-sectional study in two populations of central Europe. *Thromb Res* 2001;103:265–73.
- [73] Meng S, Ciment S, Jan M, et al. Homocysteine induces inflammatory transcriptional signaling in monocytes. *Front Biosci (Landmark edition)* 2013;18:685–95.
- [74] Li T, Yu B, Liu Z, et al. Homocysteine directly interacts and activates the angiotensin II type I receptor to aggravate vascular injury. *Nat Commun* 2018;9:11.
- [75] Yang Z, Shi J, He Z, et al. Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID-19) patients. *Aging (Albany NY)* 2019;12(2020):6037–48.
- [76] Singh Y, Gupta G, Kazmi I, et al. SARS CoV-2 aggravates cellular metabolism mediated complications in COVID-19 infection. *Dermatol Ther* 2020:e13871.
- [77] Bouvet M, Debarnot C, Imbert I, et al. In vitro reconstitution of SARS-coronavirus mRNA cap methylation. *PLoS Pathog* 2010;6:e1000863.
- [78] Liu X, Wang X-J. Potential inhibitors for 2019-nCoV coronavirus M protease from clinically approved medicines, bioRxiv (2020), p. 2020.2001.2029.924100.
- [79] Kandeel M, Al-Nazawi M. Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease. *Life Sci* 2020;251:117627.
- [80] O. Sekiou, B. Ismail, B. Zihad and D. Abdelhak, In-Silico Identification of Potent Inhibitors of COVID-19 Main Protease (Mpro) and Angiotensin Converting Enzyme 2 (ACE2) from Natural Products: Quercetin, Hispidulin, and Cirsimaritin Exhibited Better Potential Inhibition than Hydroxy-Chloroquine Against COVID-19 Main Protease Active Site and ACE2 (2020).
- [81] Narayanan N, Nair DT. Vitamin B12 May Inhibit RNA-Dependent-RNA Polymerase Activity of nsp12 from the SARS-CoV-2 Virus. Preprints 2020, 2020030347 (doi: 10.20944/preprints202003.0347.v1). Internet: <https://www.preprints.org/manuscript/202003.0347/v1>. Accessed on 16 Aug 2020.
- [82] Yu CI, Chiang B-L. A new insight into hepatitis C vaccine development, *J Biomed Biotechnol* 2010 (2010), pp. 548280-548280.
- [83] Lott WB, Takyar SS, Tuppen J, et al. Vitamin B12 and hepatitis C: molecular biology and human pathology. *Proc Natl Acad Sci U S A* 2001;98:4916–21.
- [84] Rocco A, Compare D, Coccoli P, et al. Vitamin B12 supplementation improves rates of sustained viral response in patients chronically infected with hepatitis C virus. *Gut* 2013;62:766–73.
- [85] Mokhtare M, Zeidabadi A, Bahardoust M, Safari S, Barati M, Agah S, Motavaf M. The efficacy of adding vitamin B12 to pegylated interferon and ribavirin treatment in Hepatitis C virus patients regarding the host and viral prognostic factors. *BMRAT* [Internet]. 28Feb.2019; 6(2):3016-2. Available from: <http://www.bmrat.org/index.php/BMRAT/article/view/524>. Accessed on 16 Aug 2020.
- [86] Chae HB, Park SM, Youn SJ. Direct-acting antivirals for the treatment of chronic hepatitis C: open issues and future perspectives. *Sci World J* 2013;2013:704912.
- [87] Tillmann HL. Vitamins? the magic bullet against hepatitis C. *Expert Rev Anti Infect Ther* 2012;10:1273–7.
- [88] Tan CW, Ho LP, Kalimuddin S, et al., A cohort study to evaluate the effect of combination Vitamin D, Magnesium and Vitamin B12 (DMB) on progression to severe outcome in older COVID-19 patients, medRxiv (2020), p. 2020.2006.2001.20112334.
- [89] 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;22:271–4.
- [90] Selhub J, Rosenberg IH. Excessive folic acid intake and relation to adverse health outcome. *Biochimie* 2016;126:71–8. <https://doi.org/10.1016/j.biochi.2016.04.010>.
- [91] Selhub J, Morris MS, Jacques PF, Rosenberg IH. Folate-vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency. *Am J Clin Nutr* 2009;89:702s–6s.
- [92] Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr* 2006;136:189–94.
- [93] Ministry of Health, Singapore. Agency for Care Effectiveness (ACE) Appropriate Care Guide. Oral glucose-lowering agents in type 2 diabetes mellitus - an update. Version current 03 July 2017. Internet: <http://www.ace-hta.gov.sg/our-guidance/oral-glucose-lowering-agents-in-type-2-diabetes-mellitus-an-update.html> (accessed 27 Mar 2019).
- [94] Muhktar M, Sherry D, Keaver L, McHugh CM. Metformin-induced B12 deficiency: still relevant today. *Irish J Med Sci (1971-)* 2020;189:409–10.
- [95] Cinemre H, Serinkan Cinemre BF, Çekdemir D, Aydemir B, Tamer A, Yazar H. Diagnosis of vitamin B12 deficiency in patients with myeloproliferative disorders. *J Investig Med* 2015;63:636–40.
- [96] Nexo E, Hoffmann-Lucke E. Holotranscobalamin, a marker of vitamin B-12 status: analytical aspects and clinical utility, *Am. J. Clin. Nutr.* 94 (2011), pp. 359s-365s.
- [97] Green R. Indicators for assessing folate and vitamin B-12 status and for monitoring the efficacy of intervention strategies, *Am J Clin Nutr* 94 (2011), pp. 666S–672S.
- [98] Luo P, Qiu L, Liu Y, et al., Metformin Treatment was associated with decreased mortality in COVID-19 patients with diabetes in a retrospective analysis, *Am. J. Trop. Med. Hyg.* (2020).
- [99] Sharma S, Ray A, Sadasivam B. Metformin in COVID-19: a possible role beyond diabetes. *Diabetes Res Clin Pract* 2020;164:108183.