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.

Nutrition 79–80 (2020) 111017

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Cohort study to evaluate the effect of vitamin D, magnesium, and vitamin B_{12} in combination on progression to severe outcomes in older patients with coronavirus (COVID-19)

Chuen Wen Tan M.R.C.P., F.R.C.Path [a,1], Liam Pock Ho F.R.C.P.A. [a,b,c,1], Shirin Kalimuddin M.B.B.S., M.R.C.P., M.P.H. [d,e], Benjamin Pei Zhi Cherng M.B.B.S., M.R.C.P. [d], Yii Ean Teh M.B.B.S., M.R.C.P.(UK) [d], Siew Yee Thien M.R.C.P.(UK) [d], Hei Man Wong M.R.C.P.(UK), M.Med(NUS) [d], Paul Jie Wen Tern M.B B.Chir [d], Manju Chandran M.D., F.A.C.P., F.A.C.E., F.A.M.S., C.C.D. [f], Jason Wai Mun Chay M.R.C.P., F.R.C.Path(UK) [b,c], Chandramouli Nagarajan F.R.C.P.(Edin), F.R.C.Path(UK) [a], Rehena Sultana M.Sc. (Statistics), MBA (Systems Management) [g], Jenny Guek Hong Low M.R.C.P.(UK), M.P.H.(US) [d,e], Heng Joo Ng M.R.C.P.(UK), F.R.C.Path(UK) [a,*]

[a] Department of Hematology, Singapore General Hospital, Singapore
[b] Department of Clinical Pathology, Singapore General Hospital, Singapore
[c] Department of Pathology, Sengkang General Hospital, Singapore
[d] Department of Infectious Diseases, Singapore General Hospital, Singapore
[e] Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore
[f] Department of Endocrinology, Singapore General Hospital, Singapore
[g] Centre for Quantitative Medicine, Duke-NUS Medical School, Singapore

ARTICLE INFO

Article History:
Received 24 June 2020
Received in revised form 25 August 2020
Accepted 1 September 2020

Keywords:
Vitamin D
Magnesium
Vitamin B_{12}
COVID-19

ABSTRACT

Objectives: The aim of this study was to determine clinical outcomes of older patients with coronavirus (COVID-19) who received a combination of vitamin D, magnesium, and vitamin B_{12} (DMB) compared with those who did not. We hypothesized that fewer patients administered this combination would require oxygen therapy, intensive care support, or a combination of both than those who did not.
Methods: This was a cohort observational study of all consecutive hospitalized patients ≥50 y of age with COVID-19 in a tertiary academic hospital. Before April 6, 2020, no patients received the (DMB) combination. After this date, patients were administered 1000 IU/d oral vitamin D_3, 150 mg/d oral magnesium, and 500 mcg/d oral vitamin B_{12} upon admission if they did not require oxygen therapy. Primary outcome was deterioration leading to any form of oxygen therapy, intensive care support, or both.
Results: Between January 15 and April 15, 2020, we identified 43 consecutive patients ≥50 y of age with COVID-19. Seventeen patients received DMB before onset of primary outcome and 26 patients did not. Baseline demographic characteristics between the two groups were significantly different by age. In univariate analysis, age and hypertension had a significant influence on outcome. After adjusting for age or hypertension separately in a multivariate analysis, the intervention group retained protective significance. Fewer treated patients than controls required initiation of oxygen therapy during hospitalization (17.6 vs 61.5%, $P = 0.006$). DMB exposure was associated with odds ratios of 0.13 (95% confidence interval [CI], 0.03–0.59) and 0.20 (95% CI, 0.04–0.93) for oxygen therapy, intensive care support, or both on univariate and multivariate analyses, respectively.
Conclusions: A vitamin D / magnesium / vitamin B_{12} combination in older COVID-19 patients was associated with a significant reduction in the proportion of patients with clinical deterioration requiring oxygen

CWT and LPH contributed equally. CWT, LPH, SK, JGL, and HJN co-wrote the manuscript. CWT, LPH, JWMC, MC, SK, JGL, and HJN were involved in the design of the study. BPZC, YET, SYT, HMW, PJWT, JGL, and SK conducted the pilot study. CWT, LPH, CN, and RG were involved in data analysis. LPH formulated the supplement combination. All authors have read and agreed with the manuscript. The authors have no conflicts of interest to declare.

*Corresponding author. Tel : +65-63266604. Fax : +65-62260237.
E-mail address: ng.heng.joo@singhealth.com.sg (H.J. Ng).

[1] Contributed equally

https://doi.org/10.1016/j.nut.2020.111017

support, intensive care support, or both. This study supports further larger randomized controlled trials to ascertain the full benefit of this combination in ameliorating the severity of COVID-19.

Introduction

The coronavirus (COVID)-19 pandemic, which began in late 2019, has raged across the globe with >20 million infections and 700 000 deaths recorded to date. A broad theme of immune hyperinflammation has emerged as a key determinant of patient outcome with uncontrolled immune response postulated as a pathophysiologic factor in disease severity. Intuitively, immunomodulation becomes an attractive potential treatment strategy. In addition to lung involvement, gastrointestinal symptoms are frequent and carry a worse prognosis [1]. Therefore, COVID-19 is a multiorgan phenomenon and it is becoming evident that appropriate systemic inflammatory control is necessary for overall survival benefit. Age >50 y, hypertension, diabetes, and coronary artery disease, are also known patient factors associated with increased severity and death [2].

Much of the current therapeutic effort is targeted at viral elimination instead of pre-emptively modulating hyperinflammation. A number of immunomodulatory agents may serve the latter role. Vitamin D, for instance, has a protective effect against respiratory tract infection [3]. Magnesium enhances vitamin D function in addition to being an antihypertensive, antithrombotic, and bronchodilator [4,5]. Vitamin B_{12} is an important modulator of gut microbiota [6]. Importantly, these compounds are generally safe and well tolerated by patients. A short course of vitamin D/magnesium/vitamin B_{12} supplements (DMB) upon diagnosis of COVID-19 could potentially exert synergistic effects to modulate host immune response, ameliorate COVID-19 severity, and reduce adverse outcomes. This study was conducted to evaluate the potential efficacy of DMB on progression of COVID-19 to severe disease.

Methods

Study design

This study was approved by the Institutional Ethics Committee of Singapore General Hospital with waiver of consent granted. We included all consecutive COVID-19 patients ≥50 y of age admitted to Singapore General Hospital, a tertiary academic hospital, between January 15 and April 15, 2020. Diagnosis required a positive severe acute respiratory syndrome coronavirus (SARS-CoV)-2 polymerase chain reaction (PCR) from nasopharyngeal or throat swab. Primary outcome was defined as the requirement of oxygen therapy when oxygen saturation fell <95% detected by pulse oximetry, intensive care unit (ICU) support, or both. As the COVID-19 situation evolved, we decided to start DMB beginning April 6, 2020 on all consecutive COVID-19 patients >50 y of age if they did not require oxygen therapy, ICU support, or both. These patients then served as the intervention group. All remaining patients >50 y of age during the study period who were not given DMB accordingly served as the control group. Therapy comprised a single daily oral 1000-IU dose of vitamin D_3 (colecalciferol), 150 mg of magnesium oxide, and 500 μg vitamin B_{12} (methylcobalamine) for ≤14 d. DMB could be discontinued if a patient subsequently deteriorated or was deemed to have recovered based on symptom resolution and two consecutive negative SARS CoV-2 reverse transcriptase PCR respiratory samples. All patients were followed through either to hospital discharge or day 30 from onset of symptoms, whichever was earlier.

Data collection

Clinical and laboratory data were collected from electronic health records in a standardized form with two investigators independently reviewing the data for accuracy.

Statistical analysis

Primary outcome requiring oxygen therapy was treated as binary data with categories "*yes*" or "*no*." Demographic and clinical characteristics were summarized with respect to intervention and control group. Continuous variables were expressed as mean/SD if normal distribution, or median (interquartile range [IQR]) if non-normal distribution, and categorical data was expressed as number counts and percentages as appropriate. Univariate and multivariable binary logistic regression was performed to find associated risk factors for primary outcome. Quantitative association from logistic regression was expressed as odds ratio (OR) with 95% confidence interval (CI). All tests were two-sided and $P < 0.05$ was considered statistically significant. Statistical software SPSS 25 (IBM, Armonk, NY, USA) was used for analysis.

Table 1
Baseline demographic and clinical characteristics and outcomes of the patients given DMB therapy and control patients

	DMB (n = 17)	Control (n = 26)	*P*-value
Baseline characteristics			
Age, y, mean (SD)	58.4 (7)	64.1 (7.9)	**0.021**
Male, n (%)	11 (64.7)	15 (57.7)	0.755
Female, n (%)	6 (35.3)	11 (42.3)	0.755
Comorbidities, n (%)	8 (47)	20 (76.9)	0.057
DM	0 (0)	6 (23.1)	0.066
Hypertension	6 (35.3)	18 (69.2)	0.058
Hyperlipidemia	5 (29.4)	15 (57.7)	0.118
CVD	1 (6)	6 (23)	0.376
Asthma/COPD	2 (11.7)	2 (7.7)	1.000
Stroke	0 (0)	2 (7.7)	0.511
Clinical features			
Normal CXR on admission, n (%)	7 (41.2)	7 (26.9)	0.507
Time from onset of symptoms to admission, d, median (IQR)	7 (1–9)	5 (3–8)	0.455
Time from onset of symptom to initiation of therapy, d, median (IQR)	7 (4–10)		
Time from admission to initiation of therapy, d, median (IQR)	1 (0–1)		
Duration of therapy, d, median (IQR)	5 (4–7)		
Treatment with Kaletra/remdesivir/hydroxychloroquine, n (respective numbers), %	3 (1/2/0), 17.6	16 (8/7/1), 61.5	
Outcome			
- Requiring oxygen therapy (including ICU support), n (%)	3 (17.6)	16 (61.5)	**0.006**
- Requiring oxygen therapy (but no ICU support), n (%)	2 (11.8)	8 (30.8)	
- Requiring ICU support, n (%)	1 (5.9)	8 (30.8)	
- Mortality, n (%)	0 (0)	0 (0)	

CVD, cardiovascular disease; CXR, chest x-ray; DM, diabetes mellitus; DMB, vitamin D, magnesium, vitamin B_{12}; ICU, intensive care unit; IQR, interquartile range.
P-values < 0.05 are highlighted in bold.

Results

Forty-three consecutive patients were identified, with 17 patients in the DMB arm and 26 in the control arm. Baseline demographic and clinical characteristics were significantly different for age between the two groups (Table 1). In the treatment arm, most patients received DMB within the first day of hospitalization with a median duration of therapy of 5 d (IQR = 4 to 7 d). Significantly fewer patients receiving DMB required subsequent oxygen therapy compared with controls (3 of 17 vs 16 of 26; $P = 0.006$; Table 1). On univariate analysis, increasing age and hypertension demonstrated significantly higher OR for oxygen therapy, whereas exposure to DMB therapy was associated with a significantly improved OR (Table 2). Multivariate analysis showed that DMB remained a significant protective factor against clinical deterioration after adjusting for age or hypertension separately.

Eight of the 16 patients who needed supplemental oxygen therapy in the control group also required further ICU support. Of the three

Table 2
Univariate and multivariate analyses of OR in developing primary outcome requiring oxygen therapy for clinical variables and DMB therapy

Unadjusted univariate analysis				Adjusted multivariate analysis			
Variable	OR for requiring oxygen therapy	95% CI	*P*-value	Variables	OR for requiring oxygen therapy	95% CI	*P*-value
DMB	0.134	0.031–0.586	**0.008**	DMB	0.195	0.041–0.926	**0.040**
				Age, y	1.131	1.006–1.271	**0.039**
Age, y	1.150	1.035–1.278	**0.009**				
				DMB	0.182	0.038–0.859	**0.031**
Hypertension	6.250	1.575–24.798	**0.009**	Hypertension	4.528	1.041–19.694	**0.044**
Male	2.800	0.765–10.246	0.120				
Comorbidities	3.173	0.811–12.416	0.097				
DM		*	0.999				
Hyperlipidemia	3.429	0.972–12.095	0.055				
CVD	8.214	0.868–77.739	0.066				
Asthma/COPD	1.294	0.165–10.150	0.806				
Stroke		*	0.999				

COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DM, diabetes mellitus; DMB, vitamin D, magnesium, vitamin B_{12}; OR, odds ratio.
P-values < 0.05 are highlighted in bold.
*Not estimable.

Table 3
Subgroup analysis removing patients <60 y of age with diabetes. Baseline demographic and clinical characteristics and outcomes of these patients given DMB therapy and control patients

	DMB (n = 8)	Control (n = 12)	*P*-value
Baseline characteristics			
Age, y, mean (SD)	65 (2.7)	66 (4.7)	0.571
Male, n (%)	4 (50)	6 (50)	1.000
Female, n (%)	4 (50)	6 (50)	1.000
Comorbidities, n (%)	6 (75)	10 (83.3)	1.000
Hypertension	5 (62.5)	9 (75)	0.642
Hyperlipidemia	4 (50)	6 (50)	1.000
CVD	1 (12.5)	2 (16.7)	1.000
Asthma/COPD	2 (25)	2 (16.7)	1.000
Stroke	0 (0)	0 (0)	Not estimable
Clinical features			
Normal CXR on admission, n (%)	2 (25)	2 (16.7)	1.000
Time from onset of symptoms to admission, d, median (IQR)	7 (1–10)	5 (3–8)	0.919
Time from onset of symptom to initiation of therapy, d, median (IQR)	7 (3–11)		
Time from admission to initiation of therapy, d, median (IQR)	1 (0–1)		
Duration of therapy, d, median (IQR)	7 (5–7)		
Treatment with Kaletra/remdesivir/hydroxychloroquine, n (respective numbers),%	2 (1/1/0), 25	7 (4/3/0), 58.3	
Outcome			
Requiring oxygen therapy (including ICU support), n (%)	2 (25)	7 (58.3)	0.197
- Requiring oxygen therapy (but no ICU support), n (%)	1 (12.5)	2 (16.7)	
- Requiring ICU support, n (%)	1 (12.5)	5 (41.7)	

COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DMB, vitamin D, magnesium, vitamin B_{12}; ICU, intensive care unit; IQR, interquartile range.

patients who required oxygen therapy in the DMB group, two were started on oxygen therapy within 24 h from initiation of DMB. The third patient required supplemental oxygen therapy after 3 d of DMB supplementation, but did not require ICU support. Among the nine patients given DMB within the first week of onset of symptoms, only one patient required oxygen therapy. This patient was one of the two cases who deteriorated within 24 h of DMB initiation. Of note, there were no side effects or adverse events directly attributable to DMB. There was no death in either group during the follow-up period.

Subgroup analysis was performed by removing patients with diabetes and <60 y of age, in order to better match the DMB and control groups (Table 3). Thirteen patients were excluded and the baseline characteristics did not differ between the two groups. One-fourth of the patients in the DMB group required oxygen therapy or ICU support compared with more than half (58%) of the controls.

Discussion

COVID-19 is now understood to be potentially life-threatening in ≤20% of patients. As the world awaits an effective vaccine, the effectiveness of various antivirals are largely muted by lack of survival benefit. Targeted therapies against cytokines and antithrombotic agents may only address the terminal events in severe cases with limited benefits. At the point of giving DMB to older patients, it became obvious that preemptive downregulation of hyperinflammation with relatively safe agents was an attractive alternate strategy. This combination was chosen based on substantial, albeit indirect evidence of their role in tempering the inflammatory response to viral infections. Vitamin D, through its effect on nuclear factor-κB and other pathways, can attenuate various proinflammatory cytokines mediating the uncontrolled cytokine storm seen in severe COVID-19 with deficiency associated with severe COVID-19 [7]. Magnesium is critical in the synthesis and activation of vitamin D, acting as a cofactor in many of the enzymes involved in vitamin D metabolism. Vitamin B_{12} is essential in supporting a healthy gut microbiome, which has an important role in the development and function of both innate and adaptive immune systems [8]. This could be pivotal in preventing excessive immune reaction [9], especially in COVID-19 patients with microbiota dysbiosis, which has been associated with severe disease [10].

Our results provide early positive evidence of an immunomodulatory approach to ameliorating severe outcomes in COVID-19. DMB-treated patients were significantly less likely to require oxygen therapy than controls. Among three DMB-treated patients with clinical deterioration, two likely deteriorated within 24 h from their underlying infection but were included in an intention-to-treat analysis. Had they been excluded on the basis of inadequate time of DMB exposure, the demonstrated benefits would have been more profound. The last patient who deteriorated was started on DMB 7 d after onset of symptoms. To benefit from its preemptive effects, patients may need to be started earlier in the infective course. The ease of administration of DMB should allow for early initiation in the primary care setting at first onset of symptoms, or as prophylaxis among high-risk contacts during outbreaks in identified clusters.

As all agents in this combination are readily available, safe, and inexpensive, DMB can benefit a large swath of the world population, especially in economically challenged countries with limited or late access to vaccines and other therapies. DMB may also exhibit a generic efficacy against other viral infections with similar pathologic mechanism.

The limitations of this study included its retrospective cohort design with the control arm being older and showing a trend of having more comorbidities, especially diabetes. However, subgroup analysis excluding these factors still demonstrated a lower proportion of the DMB group requiring oxygen therapy or ICU support than the controls (25 versus 58%, respectively). The benefits of DMB were retained, although statistical significance could not be demonstrated, considering the small number of patients in this subgroup analysis. Post hoc estimation of necessary sample size was performed using two-sided Fisher's exact test with assumed proportion of primary outcomes of requirement of oxygen therapy, ICU support, or both in the DMB and control groups to be 40% and 80%, respectively and the significance level of the test set as 5%. The necessary sample size was estimated to be 28 patients for both the DMB and control groups, requiring 56 patients. This study was conducted under difficult dynamic circumstances and is thus limited by the small sample size, which was smaller than our post hoc estimation. Additionally, we were not able to include systematic biological measures to support our findings.

Nonetheless, this proof-of-principle effort has yielded promising results supporting the benefit of the vitamin D / magnesium / vitamin B_{12} combination in preventing clinical deterioration in patients at high risk. Our findings will need to be further validated in a well-designed randomized controlled trial.

Acknowledgments

The authors acknowledge the continued support from SingHealth and Singapore General Hospital. They also acknowledge the doctors and nurses who care for the patients in the isolation wards at Singapore General Hospital.

References

[1] Pan L, Mu M, Yang P, Sun Y, Wang R, Yan J, et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. Am J Gastroenterology 2020;115:766–73.
[2] Verity R, Okell LC, Dorigatti I, Wiskill P, Whittaker C, Imai C, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis 2020;20:669–77.
[3] Martineau AR, Jolliffe DA, Hooper RI, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systemic review and meta-analysis of individual participant data. BMJ 2017;356:i6583.
[4] Dai Q, Zhu X, Manson JE, Song Y, Li X, Franke AA, et al. Magnesium status and supplementation influence vitamin D status and metabolism: results from a randomized trial. Am J Clin Nutr 2018;108:1258–69.
[5] Shechter M, Merz CN, Paul-Labrador M, Meisel SR, Rude RK, Molloy MD, et al. Oral magnesium supplementation inhibits platelet-dependent thrombosis in patients with coronary artery disease. Am J Cardiol 1999;84:152–6.
[6] Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a modulator of gut microbial ecology. Cell Metab 2014;20:769–78.
[7] Lau FH, Majumder R, Torai R, Saeg T, Hoffman R, Cirillo JD, et al. Vitamin D insufficiency is prevalent in severe COVID-19. MedRxiv 2020. Available at: https://doi.org/10.1101/2020.04.24.20075838. Accessed September 15, 2020.
[8] Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN. Potential role of gut microbiota in induction and regulation of innate immune memory. Front Immunol 2019;10:2441.
[9] Dhar D, Mohanty A. Gut microbiota and Covid-19—possible link and implications. Virus Res 2020;285:198018.
[10] Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization [Epub ahead of print]. Gastroenterology 2020.