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The Possible Role of Vitamin D in Suppressing Cytokine Storm and Associated Mortality in COVID-19 Patients

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Abstract

Background

Large-scale data show that the mortality of COVID-19 varies dramatically across populations, although the cause of these disparities is not well understood. In this study we investigated whether severe COVID-19 is linked to Vitamin D (Vit D) deficiency.

Method

Daily admission, recovery and deceased rate data for patients with COVID-19 from countries with a large number of confirmed patients (Germany, South Korea (S. Korea), China (Hubei), Switzerland, Iran, UK, US, France, Spain, Italy) as of April 20, 2020 were used. The time-adjusted case mortality ratio (T-CMR) was estimated as the number of deceased patients on day N divided by the number of confirmed cases on day N-8. The adaptive average of T-CMR (A-CMR) was further calculated as a metric of COVID-19 associated mortality in different countries. Although data on Vit D level is not currently available for COVID-19 patients, we leveraged the previously established links between Vit D and C-Reactive Protein (CRP) and between CRP and severe COVID-19, respectively, to estimate the potential impact of Vit D on the reduction of severe COVID-19.

Findings

A link between Vit D status and COVID-19 A-CMR in the US, France, and the UK (countries with similar screening status) may exist. Combining COVID-19 patient data and prior work on Vit D and CRP levels, we show that the risk of severe COVID-19 cases among patients with severe Vit D deficiency is 17.3% while the equivalent figure for patients with normal Vit D levels is 14.6% (a reduction of 15.6%).

Interpretation

Given that CRP is a surrogate marker for severe COVID-19 and is associated with Vit D deficiency, our finding suggests that Vit D may reduce COVID-19 severity by suppressing cytokine storm in COVID-19 patients. Further research is needed to account for other factors through direct measurement of Vit D levels.

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1. Introduction

The recent global outbreak of COVID-19 imposed catastrophic impacts on every society, specifically among elderly populations. Currently, no treatment or vaccine has been produced. Consequently, there is a significant need to elucidate potential approaches that can reduce the number of severe COVID-19 cases and thus reduce the mortality rate of the disease. It has also been proposed that the immune system in some patients may manage COVID-19 better than in others.

Time series analysis of the number of confirmed, deceased and recovered cases reveal patterns of how COVID-19 has impacted different populations, which may help improve understanding of the immune system's defense mechanisms against COVID-19 and aid in developing effective treatments for the viral infection. Data show that the mortality rate of COVID-19 varies dramatically across countries. For example, a higher case fatality ratio has been reported in Spain, Italy, and the UK compared to that of the US and Germany. The cause for these disparities is not well understood. Several hypotheses have been proposed, including the circulation of different strains of the virus [1–3], idiosyncrasies in COVID-19 testing strategies and policies across countries, quality and access to health care, demographic factors such as the prevalence of elderly within a given population, and socioeconomic factors [4]. Some experts have suggested an analysis of age-specific case fatality ratio (CFR) and time-adjusted case mortality ratio (T-CMR) for a more insightful study of COVID-19 infection [5,6]. Initial reports and data obtained from various studies suggest that the elderly have disproportionately been impacted by COVID-19 [7]. The substantially higher CFR of the elderly population thus compels an age-specific analysis of COVID-19 data.

Aging can lead to a weakening of the innate immune system [8] which may play a role in the development of severe COVID-19. Specifically, a weak innate immune system response in the elderly can lead to a higher load of COVID-19 and a consequent overactivation of the adaptive immune system, with a high level of cytokine production [9]. Clinical data obtained from COVID-19 patients in China showed high concentrations of cytokines such as GCSF, IP10, MCP1, MIP1A, and TNFα in patients admitted to the ICU, which suggests the presence of cytokine storm in these cases [10].

The role of Vit D in regulating the immune system is supported by multiple studies [11]. Vit D can suppress cytokine production by simultaneously boosting the innate immune system and avoiding the overactivation of the adaptive immune system to immediately respond to viral load. Some researchers have suggested the potential role of Vit D in suppressing cytokine storm during the 1918-1919 viral influenza pandemic [12]. Moreover, the impact of Vit D in enhancing immune response (including in flu and previous coronaviruses) has been established [11,13]. It is this ability to suppress cytokine production [14,15] that motivated our focus on Vit D deficiency and its association with severe COVID-19.

To the best of our knowledge, no randomized blinded experiment has yet reported Vit D status and cytokine levels in patients with COVID-19. Despite this, it is possible to estimate the association between Vit D status and severe COVID-19 based on a potential link between Vit D deficiency and C-reactive proteins (CRP) [16]. CRPs are produced by the liver in response to inflammation to minimize damage to arteries, cells, and tissue

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from autoimmunity, infection, and other causes. The inflammatory cells' ability to convert Vit D metabolites into calcitriol (the active form of Vit D) and to express the nuclear receptor of Vit D suggests a potential inverse association between CRP and Vit D, which is also supported by epidemiological studies [17,18]. Early studies have shown that calcitriol treatment attenuates both CRP and inflammatory cytokines (CD4(+) IFN-γ) in hemodialysis patients [19]. Researchers have proposed that calcitriol modulates cytokine levels (such as TNF-α and IL-1β) using the intercellular role of calcium [20,21].

Below we combine data from studies of Vit D and CRP [16] with data from COVID-19 patients [22] to investigate the potential role of Vit D in severe COVID-19.

2. Methods

Data regarding the number of affected cases, deaths, and recoveries from COVID-19 was obtained from Kaggle [23] as of April 20, 2020. Data regarding testing cases was obtained from Our World in Data [32]. Age distribution of confirmed cases, those admitted to ICU, and deceased patients in Spain was based on data available from the Spanish Ministry of Health [24]. The concentration of 25(OH)D among the elderly population in each country was obtained from prior studies [25–30]. Data on the link between Vit D and CRP were taken from a nationwide cross-sectional dataset from the National Health and Nutrition Examination study in the US [16]. The link between CRP and severe COVID-19 was examined based on data from a study investigating the characteristics of COVID-19 patients in China [22]. The T-CMR is defined as the estimated ratio of deceased patients on day N (D_N) to confirmed patients on day N-8 (C_{N-8}). Adaptive averaging of T-CMR (A-CMR) was calculated based on a weighted average technique as shown in Equation (1).

$$\text{A-CMR} = \sum_{n=1}^{n=N} a_n \times \text{T-CMR [n]}, \quad a_n = c_n / \sum_{i=1}^{i=N} c_i \ , \tag{1}$$

where N is the number of days with more than 10,000 confirmed cases in the country (except in S. Korea where the threshold is 5,000), c_i is the number of confirmed cases at day i, T-CMR (n) is T-CMR on day n, and a_n is a coefficient that describes the weight of T-CMR on day n. Positivity change (PC) is calculated using a moving average of size 5 on the ratio of new daily confirmed cases to the new daily tested individuals on day N as shown by Equation (2).

$$PC = \sum_{i=1}^{i=5} 0.2 \times (C_{N+1-i} - C_{N-i}) / (T_{N+1-i} - T_{N-i}), \tag{2}$$

where C_N is the total confirmed cases on day N and T_N is the total number of tested cased on day N. Risks and conditional risks of the events were estimated using the ratio of the number of events in a treated group to the number of patients in the group.

3. Results and Interpretation

3.1. COVID-19 Fatality

Ambiguity on the incubation period of COVID-19 makes the calculation of the true mortality rate for COVID-19 a challenging task [5,6]. Bureaucratic screening policies, as well as demographic and cultural variables further diminish the possibility of estimating disease onset and calculating an accurate case mortality rate (CMR). Analysis of time events reported from 41 deceased patients in Wuhan (Hubei, China) shows a median time of 8 days between admission and time of death, and 14 days between the onset of

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symptoms and time of death (shown in the inset in Figure 1 (a)) [31]. This suggests a delay between the time the confirmed cases are reported and the time deceased patients are counted. In other words, the total number of deceased patients at day N (D_N) is attributed to the total number of confirmed patients at day N-8 (C_{N-8}) which is equal to the total number of cases at the onset of the symptoms on day N-14 (O_{N-14}). Time adjusted-CMR (T-CMR) with a delay of 8 days (D_N/ C_{N-8}) is therefore used in this study (shown in Figure 1(a)). Calculating the percent difference between T-CMR on April 20 and April 6 for three different delays of 0 days, 8 days and 14 days suggests an 8-day delay presents the least variation across countries. This analysis acknowledges that the calculation of T-CMR with an 8-day delay is less sensitive to an abrupt change in the number of confirmed/deceased patients in a single day for a given country. Figure 1(a) shows time series data for T-CMR drifting for some countries. Intense variations in the ratio of confirmed to tested patients can change the results for T-CMR over the pandemic for multiple reasons. With the deaths of the most vulnerable members of a population, T-CMR is expected to decrease over time. In addition, increasing screening capabilities, will increase the chance of identifying mild cases thus reducing T-CMR. As a result, different values for T-CMR are calculated throughout the pandemic, and the question arises of which value is more representative of the intrinsic mortality characteristic of the virus within each country.

A-CMR

In countries with a wide variation in confirmed cases, T-CMR varies each day and this increases the uncertainty in the actual T-CMR within the country. To calculate a more accurate estimate of T-CMR we created a framework based on two hypotheses. First, we considered only outbreaks of 10,000 confirmed patients or greater (except in S. Korea where the threshold was 5,000) to provide a reliable T-CMR. Next, an average of the T-CMRs was calculated given a higher weight for the T-CMRs that represent a higher population. A-CMR for each country is calculated using Equation (1) and the results (shown in Figure 1 (b)) suggest varying A-CMR values across countries.

S. Korea and Germany report a comparably low A-CMR of 1.8% and 3.1%. The A-CMR in Switzerland (A-CMR = 5.3%) and China (A-CMR = 5.5%) is higher than in S. Korea and Germany but is lower than in the US (A-CMR = 8%) and Iran (A-CMR of 9.8 %). Spain (A-CMR = 17.5%), Italy (A-CMR = 18.6%), France (A-CMR = 20.7%) and the UK (A-CMR = 24.5%) report the highest A-CMR. Multiple factors may contribute to the difference in A-CMR across these countries. Figure 1 (c) shows the average ratio of confirmed (C) to tested (T) cases in each country. Comparison of Figure 1(b) and 1(c) shows that countries with mass screening policies (low C/T ratio) report a substantially lower A-CMR than other countries. One reason could be countries with an aggressive screening policy tend to detect more cases of mild COVID-19 and will thus report a lower A-CMR, as mild COVID-19 cases are generally not fatal. In addition, a slow growth rate of confirmed patients may potentially cause a different and less fatal version of the virus to circulate across countries as the more fatal version of the virus is contained in hospitals. When the virus is spreading across the country with a fast growth rate, more fatal versions of the virus have more ways and likelihood of spreading, which may result in an increase of A-CMR in a country. We consider positivity (C/T) or PC to be a better indicator of the impact of screening policy than total tests per capita. The reason is that a low number of

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tests per capita can be used when the total number of patients is low, but as the number of patients increases substantially higher tests per capita are required to facilitate detection of mild COVID-19 cases.

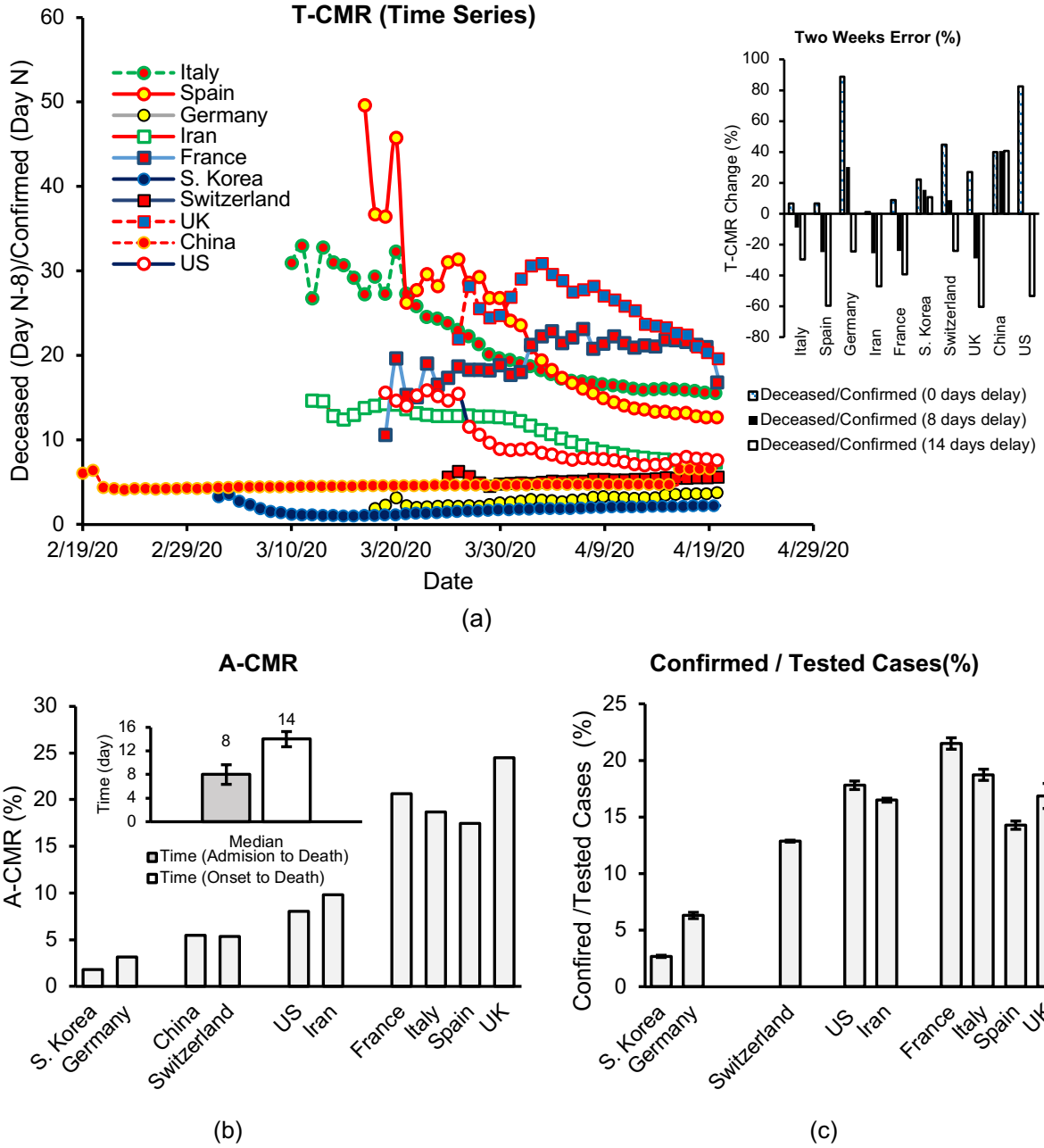

Figure 1 (a) The T-CMR (8 days) as of April 20. A two week error (100 × (T-CMR$_{\text{April 20}}$ – T-CMR$_{\text{April 6}}$)/ T-CMR$_{\text{April 6}}$) calculated at different T-CMR delays of 0 days, 8 days and 14 days. (b) A-CMR as of April 20 [23]. (C) Percentage of confirmed to tested ratio suggests an impact of screening policies in different countries on A-CMR [23,32–35]. France has reported the number of tests [32]. The UK data includes reported number of tests (from April6-April 20) and estimated number tests by multiplying the number of tested patients by 1.26 (estimated from the relation between the number of tests and patients after April 6-April 20) [32]. US data is mainly the number of people tested (some labs have reported the number of tests) [32]. The number of tests conducted in Iran and Spain is estimated from two reported statements by public authorities [32–35].

Age distribution of the population is also important factor that impacts mortality for a given country as COVID-19 has shown to be deadlier in elderly patients [7]. In the following, information obtained from PC and prevalence is used to conduct a more in-depth analysis of screening strategies in different countries.

Screening Status

It is important to control for screening strategies and age distribution across countries before comparing Vit D status as such variables may notably impact A-CMR. Two factors can be used to evaluate the screening status in different countries; 1) PC, and 2) the prevalence of the COVID-19. We first calculated the PC to provide an illustration of the variation in positivity in different countries over time in Figure 2. The average PC value in the first 14 days is calculated and the results are shown in the inset of Figure 2. Based on this analysis, we observed that S. Korea, Germany, and Switzerland have lower PC values consistently, while Iran, the US, France, Italy, Spain, and the UK share higher PC values. The starting point of each curve is the day that the country reported at least 10,000 patients in total (except S. Korea > 5,000).

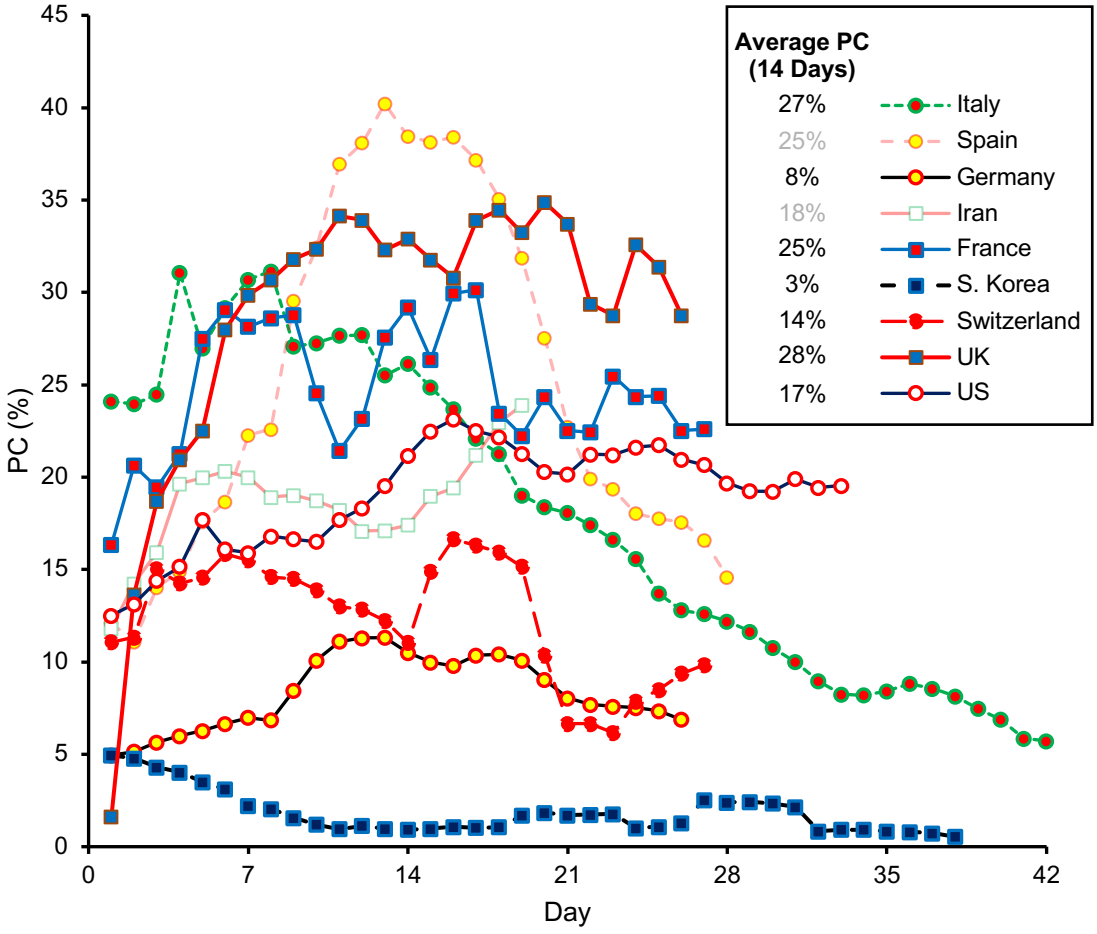

Figure 2- PC over time compares growth rate of COVID-19.

A weakness in this analysis is that positivity depends on the prevalence of COVID-19. Next, we advanced our analysis by evaluating PC as a function of prevalence. We

calculated an average number of confirmed cases per 1 million population per day in 21 days (r_c) and used it as an indicator of the prevalence of COVID-19 in each country. We plotted the PC against r_c for two weeks in Figure 3 and the results suggest the countries are divided into two groups where a more aggressive screening strategy is used in S. Korea, Germany and Switzerland compared to Spain, Italy, France, the UK, the US, and Iran. The initial state of each curve is similar to the one in Figure 1 and Figure 2 where at least 10,000 confirmed patients (except 5,000 for S. Korea) are used to start the analysis. A testing aggressiveness index (TAI) is calculated using Equation (3) which presents a quantitative illustration for Figure 3.

$$TAI = \sum_{n=1}^{n=14} r_c[n] / PC[n],\ r_c[n] = (C_n - C_{n-21})/ P \quad (3)$$

Where P is the population in millions of the countries, C_n is the total number of confirmed patients on day n. TAI values for each country are presented in the inset in Figure 3.

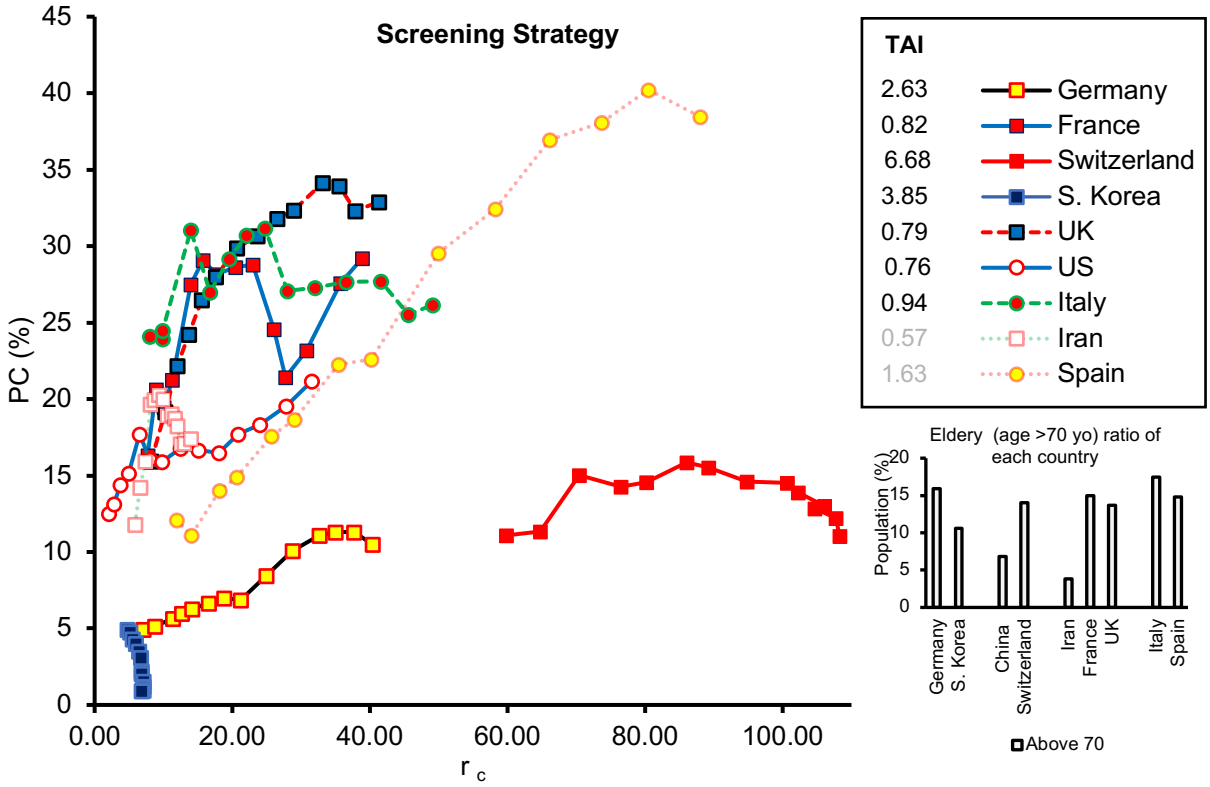

Figure 3- PC against r_c for two weeks after each country reaches 10,000 patients (except S. Korea >5,000 patients).

A small TAI is associated with a large delta in PC with a small delta in prevalence which indicates the population of the tested subjects (associated with PC) does not represent the population of confirmed subjects across the country's population (associated with prevalence), indicating a less aggressive screening strategy. The quantitative illustration of TIA suggests a more aggressive screening status (2.60<TIA<6.70) in Germany, S. Korea, and Switzerland, while a less aggressive screening status in Spain, Italy, France, the UK, the US, and Iran (0.55<TIA<1.65). The least aggressive screening status is suggested by Iran with TIA of 0.57. It needs to be noted that Spain and Iran have reported a complete daily confirmed patients' information however they have reported limited data

about their testing cases. The screening data from Iran and Spain are estimated from only two testing data points with an average new daily test rate reported by the public authorities. The limited number of data points may increase the error in our estimation, which is why their presented results are highlighted in gray instead. Information about all the countries' age distributions is shown in the inset of Figure 3 suggest a similar age distribution between the US, the UK, France, Spain and Germany.

Possible Effect of Vit D on A-CMR

The screening status and the age distribution can notably impact A-CMR in a population of a given country. To evaluate the possible association of A-CMR with Vit D we need to ensure that both the screening status and age distribution of the elderly are similar between targeted countries that are being compared.

Countries with Less Aggressive Screening Status

The 25(OH)D concentration among the elderly (age>60 yo or age>65 yo) in countries with less aggressive screening policies are shown in Figure 4 (a). A comparison of the A-CMR and the mean 25(OH)D concentration among the elderly suggests an inverse relationship between the two. In particular, the UK, with the lowest mean 25(OH)D level, reports the highest A-CMR while the US with the highest mean 25(OH)D reports the lowest A-CMR. Iran and France, countries with higher mean 25(OH)D concentration than the UK, report a lower A-CMR. The age distribution of the elderly among these countries, shown in the inset in Figure 3, indicates the US, France, and the UK have a similar elderly distribution while Iran and China have a lower elderly population than others.

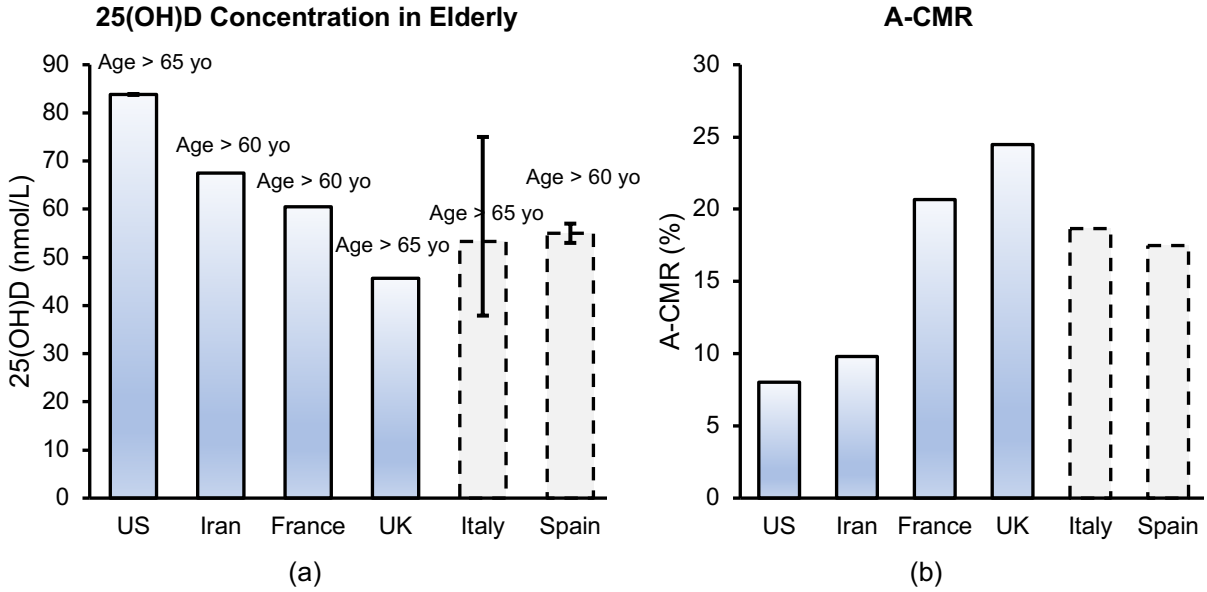

Figure 4- (a) Mean 25(OH)D in the elderly population in the US [36], Iran [37], France [38] and the UK [39], Average of three reported median 25(OH)D for Italy [25–27], Average of two reported median 25(OH)D among elderly Spain. Median of 25(OH)D in Spain has been estimated from a Figure in the manuscript . Error bar shows the range of reported median 25(OH)D in a different study [28]. (b) A-CMR for the US, Iran, France, UK, Italy and Spain (countries with less aggressive screening status).

An estimate of 25(OH)D concentration from Italy and Spain is also included in this figure. Unfortunately, since we were unable to determine the mean 25(OH)D concentration for Italy and Spain, thus, an average for the reported median of 25(OH)D concentration from different studies has been provided with a dashed line for these two countries [25–28]. Studies involving different cohorts (the Asturias study and the Pizarra study) in Spain estimated a slightly different concentration of 25(OH)D among the Spanish population. This led us to expect a median concentration between 53nmol/L to 59.5 nmol/L (values estimated from a figure) [28]. The variation of reported 25(OH)D concentration of the elderly population in Italy was concerning. A study of 13,110 adults in Northwestern Italy estimated the median 25(OH)D concentration of 47 nmol/L among the elderly living there [25], while another study using data from 2,694 community-dwelling elderly from Northern Italy (results from the Progetto Veneto Anziani study) estimated a median of 75 nmol/L [26]. A third study of 697 elderly women in southern Italy estimated a mean 25(OH)D concentration of 37.9 nmol/L [27].

Countries with Aggressive Screening Status

Based on our analysis, Germany and S. Korea appear to have an aggressive screening strategy (Figures 2 & 3). The mean 25(OH)D concentration and A-CMR (shown in Figure 5) indicate that S. Korea is reporting a lower A-CMR than Germany while also reporting a higher mean 25(OH)D among the elderly. Although sensitivity analysis comparing Vit D status in the elderly population in countries with similar screening status suggests a possible role of Vit D deficiency of the elderly population in affecting A-CMR, more countries should be compared to better substantiate this analysis.

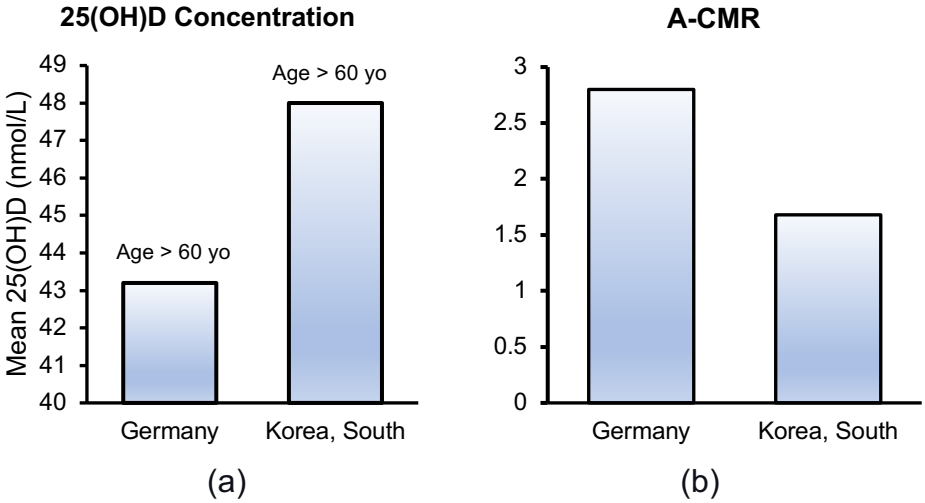

Figure 5- Mean 25(OH)D concentration in the elderly population in Germany [29] and S. Korea [30].

3.2. Disparity in Confirmed, Hospitalized and Admitted to ICU Cases across Age Groups

The impact of aging on innate immunity may influence the body's response against COVID-19. Figure 6 shows the age distributions of patients who were hospitalized, admitted to the ICU, and deceased based on 145,429 cases from Spain. It shows COVID-

19's alarming impact on the elderly. In particular, 61% of the patients above 70 yo were hospitalized and 20% died. Other studies have shown a similar vulnerability to COVID-19 among elderly populations [41][42][43][44]. The results shown in Figure 6 suggest a higher risk of hospitalization and admission to the ICU for elderly patients. A possible explanation for this is that a weak innate immune system response to COVID-19 can allow a high viral load, which then leads to a higher rate of complications and hospitalization. Consequent overactivation of the adaptive immune system and high levels of cytokine production [9] could then lead to complications that must be addressed in the ICU. A notably higher ratio of deceased to ICU-admitted patients among the elderly suggests that the impact of cytokine storm is more severe, and the threshold for tolerating its side effects is lower, for elderly patients. Figure 6 (b) also shows a higher ratio of admission to the ICU for children (<4 yo) which could be due to a relatively immature immune system [45]. A very low fatality rate among children suggests a higher threshold for tolerating complications associated with COVID-19 than among the elderly.

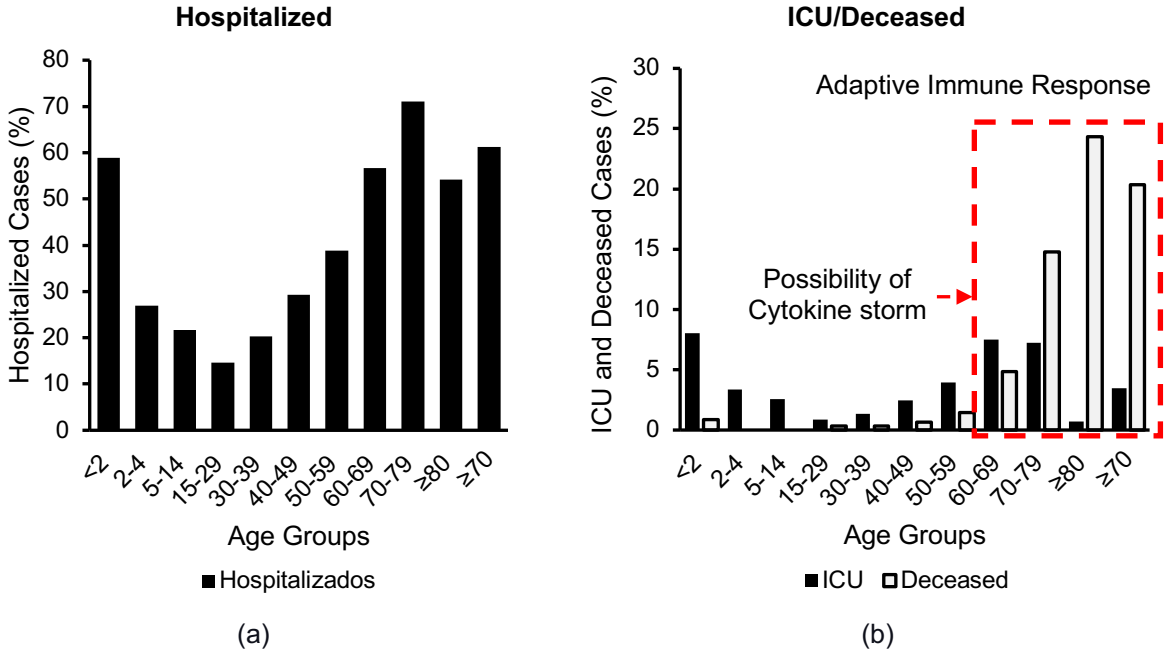

Figure 6- Age distribution of the a) hospitalized, b) admitted to ICU or deceased in Spain based on data from 145,429 cases [24].

3.3. CRP and Severe COVID-19

Table 1 shows the risks of severe and mild COVID-19 under different CRP levels, based on clinical data from 793 confirmed COVID-19 patients in China (up to 52 hospitals in 30 provinces) [22]. According to this dataset, patients with severe COVID-19 have a higher percentage of cases with CRP ≥ 10 mg (81.5%, 110 cases out of 135) than those with a mild form of the disease (56.5%, 371 cases out of 658). Conversely, patients with high CRP have a higher risk of severe COVID-19 (23%) than their counterparts with low CRP (8%). This trend also persists in the cases of mild COVID-19.

Table 1. The risks of severe and mild COVID-19 under different CRP levels, based on data reported by [22].

	Number of Events/Total Patients (Risk)
Risk of High CRP	481/793 (61%)
Risk of Low CRP	312/793 (39%)
Risk of High CRP given Severe COVID-19	110/135(81%)
Risk of Low CRP given Sever COVID-19	25/135(19%)
Risk of Severe COVID-19 given High CRP	110/481 (23%)
Risk of Severe COVID-19 given Low CRP	25/312 (8%)
Risk of High CRP given Mild COVID-19	371/658(56%)
Risk of Low CRP given Mild COVID-19	285/658(44%)

3.4. CRP and Vit D Deficiency

The relationship between CRP and Vit D has been investigated in multiple clinical studies. Laboratory data from 1,873 participants (NHANES, 2007-2008) and analyzed by Li et al. shows a strong relationship between severe deficiency of Vit D and high levels of CRP [16]. Using this dataset, Table 2 estimates the risks of high CRP for different Vit D levels. We observed a risk factor of 1.4 for high CRP plasma in subjects with severely deficient Vit D levels (25(OH)D < 25nmol/L). Put another way, subjects with a severe deficiency of Vit D have a 1.4 times higher risk of high CRP.

Table 2. Risk of high CRP given Vit D status based on the data reported in [16].

25(OH)D (nmol/L)	High CRP/Low CRP (Risk of High CRP)
at Normal Vit D (>75)	495/597 (45%)
at Insufficient Vit D (50-75)	729/717 (50%)
at Deficient Vit D (25-50)	639/476 (57%)
at Severely Deficient Vit D (<25)	122/73 (63%)

3.5. Risk of Severe COVID-19

Because high CRP (a surrogate of cytokine storm) levels are associated with severe COVID-19 and severe Vit D deficiency is associated with high CRP, we can estimate the extent to which eliminating Vit D deficiency may lower the risk of severe COVID-19. Conditional risk of severe COVID-19 given the severe deficiency of Vit D or given a normal Vit D status can be calculated using Equation (4).

$$\text{Risk (Severe COVID-19|Vit D Status)} = \text{Risk (High CRP|Vit D Status)} \times \text{Risk (Severe COVID-19|High CRP)} + \text{Risk (Low CRP|Vit D Status)} \times \text{Risk (Severe COVID-19|Low CRP)} \quad (4)$$

We found that the Risk (Severe COVID-19|Severe Deficient Vit D) = 63% × (110/481) + 37% × (25/312) = 17.4% while the Risk (Severe COVID-19|Normal Vit D) = 45% × (110/481) + 55% × (25/312) = 14.7%. This calculation suggests the potential for a 15.6% reduction in the risk of severe COVID-19 cases by eliminating severe Vit D deficiency.

4. Discussion

Our findings suggest that Vit D deficiency may be a contributing factor to severe COVID-19. One possible explanation for this association is that the weak response of the innate immune system in the elderly may increase viral load [8,46] while a shortage in memory B cells leads to misfire and over-activation of the adaptive immune system by producing a high level of cytokines, or a cytokine storm. These processes are exacerbated by low levels of Vit D. On the other hand, Vit D plays a role in enhancing the innate immune system while, at the same time, partially suppressing adaptive immunity and some of its complications such as the induction of cytokine storm. In turn, cytokine storm [47] may instigate further complications such as ARDS, exacerbation of the effects of pneumonia, acute kidney failure, acute heart failure, and rhabdomyolysis [22] that in some cases may become fatal. Even moderate lung damage due to a weak cytokine storm could lead to hypoxemia that in turn results in mortality due to underlying conditions.

Of particular note is that the time interval for the development of a substantial adaptive immune response, which has been estimated to be approximately 7 days [48], is consistent with the time course of COVID mortality [31]. Differentiation of CD8+ T cells into cytotoxic T lymphocytes (CTLs) by the adaptive immune system [49] may initiate the production of cytokines after about 7 days. Overproduction of cytokines can necessitate the transfer of the patient to the ICU or become fatal in the next few weeks [50]. The reported potential role of ibuprofen in worsening COVID-19 treatment [51] might also be partially explained by its suppression of innate immunity [52,53] which leads to a higher viral load and consequent overactivation of the adaptive immune system which again may become fatal in elderly patients [54,55]. Vit D, on the other hand, may boost the innate immune system and suppress the adaptive immune system, thus lowering cytokine levels [11,13]. Therefore the role of Vit D in regulating immunity and suppression of cytokine storm may help avoid potential complications in elderly and African-American patients as these individuals are more likely to experience a severe Vit D deficiency [8].

One important limitation of the present country-level analysis is the assumption that Vit D levels in COVID-19 patients follow the same distribution with subjects in previous Vit D studies. In addition, the difference in age range, ethnicity, gender, social status, geographic latitude, the season of sample collection, and year of study may impact the reported value of Vit D status in different studies. Analysis of data on Vit D levels and cytokine levels from individual patients with COVID-19 may reveal stronger evidence for this link. The intrinsic cross-sectional nature of this study does not prove a relationship between Vit D, CRP levels, cytokine storm, and severe COVID-19. Vit D data have been collected from different sources and variation between and within different studies introduces variations in the data. Another important limitation of this study is that crude mortality data is used instead of age-specific mortality data. This is because the age distribution of confirmed patients in the US and UK were not available. The onset of COVID-19 for confirmed cases is unknown and is assumed to be similar for all subjects. In addition, other underlying conditions associated with the populations at risk of Vit D deficiency makes it more challenging to assess the actual impact of Vit D versus other factors. Finally, our data suggest differences in the COVID-19 testing strategies and policies across countries. This makes an accurate assessment of mortality difficult. To address this issue, in our analysis we grouped countries based on the presumptive

similarities between their testing strategies. These limitations can be addressed by following Vit D and COVID-19 status in individual patients within a given population. Such data, however, is currently unavailable. The link between Vit D and the probability of severe COVID-19 and associated mortality that is indicated by this work may serve as an impetus for such studies.

5. Conclusion

Large-scale data shows that different countries have differing A-CMR among confirmed cases. Screening status notably impacts A-CMR, as countries with aggressive COVID-19 screening show decreased A-CMR. A sensitivity analysis across countries with similar screening status and age distribution (e.g. US, France, and the UK) suggests that Vit D may have an effect on A-CMR. Our preliminary analysis of COVID-19 patient data [22] combined with a Vit D research study [16] suggests that Vit D may reduce COVID-19 fatality by suppressing cytokine storm [10,14,21]. Specifically, the risk of severe COVID-19 cases among patients with severe Vit D deficiency is 17.3% while the equivalent figure for patients with normal Vit D level is 14.6% (a reduction of 15.6%). This potential effect may be attributed to Vit D's ability to suppress the adaptive immune system, regulating cytokine level and thereby reducing the risk of developing severe COVID-19. For more accurate estimates, future work needs to account for more factors and to collect patient-level data, particularly regarding Vit D levels.

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References

[1] X. Tang, C. Wu, X. Li, Y. Song, X. Yao, X. Wu, Y. Duan, H. Zhang, Y. Wang, Z. Qian, J. Cui, J. Lu, On the origin and continuing evolution of SARS-CoV-2, Natl. Sci. Rev. (n.d.). https://doi.org/10.1093/nsr/nwaa036.

[2] H. Yao, X. Lu, Q. Chen, K. Xu, Y. Chen, L. Cheng, F. Liu, Z. Wu, H. Wu, C. Jin, M. Zheng, N. Wu, C. Jiang, L. Li, Patient-derived mutations impact pathogenicity of SARS-CoV-2, MedRxiv. (2020) 2020.04.14.20060160. https://doi.org/10.1101/2020.04.14.20060160.

[3] Coronavirus has mutated into 30 strains and ones in US are less deadlier than those in Europe, finds study, News Break. (n.d.). https://www.newsbreak.com/news/0OoSRJjb/coronavirus-has-mutated-into-30-strains-and-ones-in-us-are-less-deadlier-than-those-in-europe-finds-study (accessed April 21, 2020).

[4] J.B. Dowd, L. Andriano, D.M. Brazel, V. Rotondi, P. Block, X. Ding, Y. Liu, M.C. Mills, Demographic science aids in understanding the spread and fatality rates of COVID-19, Proc. Natl. Acad. Sci. (2020). https://doi.org/10.1073/pnas.2004911117.

[5] Mortality: Statistics, (2016) 572–577. https://doi.org/10.1016/B978-0-12-800034-2.00297-4.

[6] D. Baud, X. Qi, K. Nielsen-Saines, D. Musso, L. Pomar, G. Favre, Real estimates of mortality following COVID-19 infection, Lancet Infect. Dis. 0 (2020). https://doi.org/10.1016/S1473-3099(20)30195-X.

[7] G. Onder, G. Rezza, S. Brusaferro, Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy, JAMA. (2020). https://doi.org/10.1001/jama.2020.4683.

[8] C.R. Gomez, V. Nomellini, D.E. Faunce, E.J. Kovacs, Innate immunity and aging, Exp. Gerontol. 43 (2008) 718–728. https://doi.org/10.1016/j.exger.2008.05.0168.05.016.

[9] P. Mehta, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall, J.J. Manson, COVID-19: consider cytokine storm syndromes and immunosuppression, The Lancet. 395 (2020) 1033–1034. https://doi.org/10.1016/S0140-6736(20)30628-0.

[10] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, The Lancet. 395 (2020) 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

[11] C. Aranow, Vitamin D and the Immune System, J. Investig. Med. Off. Publ. Am. Fed. Clin. Res. 59 (2011) 881–886. https://doi.org/10.231/JIM.0b013e31821b8755.

[12] The possible roles of solar ultraviolet-B radiation and vitamin D in reducing case-fatality rates from the 1918-1919 influenza pandemic in the Unit... - PubMed - NCBI, (n.d.). https://www.ncbi.nlm.nih.gov/pubmed/20592793 (accessed April 5, 2020).

[13] N. Goncalves-Mendes, J. Talvas, C. Dualé, A. Guttmann, V. Corbin, G. Marceau, V. Sapin, P. Brachet, B. Evrard, H. Laurichesse, M.-P. Vasson, Impact of vitamin d supplementation on influenza vaccine response and immune functions in deficient elderly persons: a randomized placebo-controlled trial, Front. Immunol. 10 (2019). https://doi.org/10.3389/fimmu.2019.00065.

[14] E. Parlak, A. Ertürk, Y. Çağ, E. Sebin, M. Gümüşdere, The effect of inflammatory cytokines and the level of vitamin D on prognosis in Crimean-Congo hemorrhagic fever, Int. J. Clin. Exp. Med. 8 (2015) 18302–18310.

[15] D. Khare, N.M. Godbole, S.D. Pawar, V. Mohan, G. Pandey, S. Gupta, D. Kumar, T.N. Dhole, M.M. Godbole, Calcitriol [1, 25[OH]2 D3] pre- and post-treatment suppresses inflammatory response to influenza A (H1N1) infection in human lung A549 epithelial cells, Eur. J. Nutr. 52 (2013) 1405–1415. https://doi.org/10.1007/s00394-012-0449-7.

[16] Q. Li, Z. Dai, Y. Cao, L. Wang, Association of C-reactive protein and vitamin D deficiency with cardiovascular disease: A nationwide cross-sectional study from National Health and Nutrition Examination Survey 2007 to 2008, Clin. Cardiol. 42 (2019) 663–669. https://doi.org/10.1002/clc.23189.

[17] K. Yin, D.K. Agrawal, Vitamin D and inflammatory diseases, J. Inflamm. Res. 7 (2014) 69–87. https://doi.org/10.2147/JIR.S63898.

[18] L.C.Y. Liu, A.A. Voors, D.J. van Veldhuisen, E. van der Veer, A.M. Belonje, M.K. Szymanski, H.H.W. Silljé, W.H. van Gilst, T. Jaarsma, R.A. de Boer, Vitamin D status and outcomes in heart failure patients, Eur. J. Heart Fail. 13 (2011) 619–625. https://doi.org/10.1093/eurjhf/hfr032.

[19] C.-C. Wu, J.-H. Chang, C.-C. Chen, S.-B. Su, L.-K. Yang, W.-Y. Ma, C.-M. Zheng, L.-K. Diang, K.-C. Lu, Calcitriol treatment attenuates inflammation and oxidative stress in hemodialysis patients with secondary hyperparathyroidism, Tohoku J. Exp. Med. 223 (2011) 153–159. https://doi.org/10.1620/tjem.223.153.

[20] V. Panichi, S. De Pietro, B. Andreini, A.M. Bianchi, M. Migliori, D. Taccola, L. Giovannini, C. Tetta, R. Palla, Calcitriol modulates in vivo and in vitro cytokine production: A role for intracellular calcium, Kidney Int. 54 (1998) 1463–1469. https://doi.org/10.1046/j.1523-1755.1998.00152.x.

[21] T. Volk, M. Hensel, K. Mäding, K. Egerer, W.J. Kox, Intracellular Ca2+ dependence of nitric oxide mediated enhancement of interleukin-8 secretion in human endothelial cells, FEBS Lett. 415 (1997) 169–172. https://doi.org/10.1016/s0014-5793(97)01117-4.

[22] W. Guan, Z. Ni, Y. Hu, W. Liang, C. Ou, J. He, L. Liu, H. Shan, C. Lei, D.S.C. Hui, B. Du, L. Li, G. Zeng, K.-Y. Yuen, R. Chen, C. Tang, T. Wang, P. Chen, J. Xiang, S. Li, J. Wang, Z. Liang, Y. Peng, L. Wei, Y. Liu, Y. Hu, P. Peng, J. Wang, J. Liu, Z. Chen, G. Li, Z. Zheng, S. Qiu, J. Luo, C. Ye, S. Zhu, N. Zhong, Clinical Characteristics of Coronavirus Disease 2019 in China, N. Engl. J. Med. 0 (2020) null. https://doi.org/10.1056/NEJMoa2002032.

[23] Novel Corona Virus 2019 Dataset | Kaggle, (n.d.). https://www.kaggle.com/sudalairajkumar/novel-corona-virus-2019-dataset (accessed April 6, 2020).

[24] Informes COVID-19, (n.d.). https://www.isciii.es/QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTransmisibles/Paginas/InformesCOVID-19.aspx (accessed April 4, 2020).

[25] M. Basile, L. Ciardi, I. Crespi, E. Saliva, G. Bellomo, M. Vidali, Assessing Serum Concentrations of 25-Hydroxy-Vitamin D in North-Western Italy, J. Frailty Aging. 2 (2013) 174–178. https://doi.org/10.14283/jfa.2013.25.

[26] E.D. Toffanello, E. Perissinotto, G. Sergi, S. Zambon, E. Musacchio, S. Maggi, A. Coin, L. Sartori, M.-C. Corti, G. Baggio, G. Crepaldi, E. Manzato, Vitamin D and Physical Performance in Elderly Subjects: The Pro.V.A Study, PLOS ONE. 7 (2012) e34950. https://doi.org/10.1371/journal.pone.0034950.

[27] S. Adami, O. Viapiana, D. Gatti, L. Idolazzi, M. Rossini, Relationship between serum parathyroid hormone, vitamin D sufficiency, age, and calcium intake, Bone. 42 (2008) 267–270. https://doi.org/10.1016/j.bone.2007.10.003.

[28] I. González-Molero, S. Morcillo, S. Valdés, V. Pérez-Valero, P. Botas, E. Delgado, D. Hernández, G. Olveira, G. Rojo, C. Gutierrez-Repiso, E. Rubio-Martín, E.

Menéndez, F. Soriguer, Vitamin D deficiency in Spain: a population-based cohort study, Eur. J. Clin. Nutr. 65 (2011) 321–328. https://doi.org/10.1038/ejcn.2010.265.

[29] M. Rabenberg, C. Scheidt-Nave, M.A. Busch, N. Rieckmann, B. Hintzpeter, G.B.M. Mensink, Vitamin D status among adults in Germany – results from the German Health Interview and Examination Survey for Adults (DEGS1), BMC Public Health. 15 (2015). https://doi.org/10.1186/s12889-015-2016-7.

[30] J.-H. Park, I.Y. Hong, J.W. Chung, H.S. Choi, Vitamin D status in South Korean population, Medicine (Baltimore). 97 (2018). https://doi.org/10.1097/MD.0000000000011032.

[31] J.T. Wu, K. Leung, M. Bushman, N. Kishore, R. Niehus, P.M. de Salazar, B.J. Cowling, M. Lipsitch, G.M. Leung, Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China, Nat. Med. (2020) 1–5. https://doi.org/10.1038/s41591-020-0822-7.

[32] To understand the global pandemic, we need global testing – the Our World in Data COVID-19 Testing dataset, Our World Data. (n.d.). https://ourworldindata.org/covid-testing (accessed April 13, 2020).

[33] Over 6,000 being tested for COVID-19 in Iran per day, Tehran Times. (2020). https://www.tehrantimes.com/news/446155/Over-6-000-being-tested-for-COVID-19-in-Iran-per-day (accessed April 21, 2020).

[34] Coronavirus testing in Europe, by country 2020, Statista. (n.d.). https://www.statista.com/statistics/1109066/coronavirus-testing-in-europe-by-country/ (accessed April 21, 2020).

[35] Spain Becomes Third Country to Report Over 20,000 Virus Deaths, Bloomberg.Com. (2020). https://www.bloomberg.com/news/articles/2020-04-18/spain-becomes-third-country-to-report-over-20-000-virus-deaths (accessed April 21, 2020).

[36] J. Wei, A. Zhu, J.S. Ji, A Comparison Study of Vitamin D Deficiency among Older Adults in China and the United States, Sci. Rep. 9 (2019) 1–11. https://doi.org/10.1038/s41598-019-56297-y.

[37] S. Hovsepian, M. Amini, A. Aminorroaya, P. Amini, B. Iraj, Prevalence of Vitamin D Deficiency among Adult Population of Isfahan City, Iran, J. Health Popul. Nutr. 29 (2011) 149–155.

[38] J.-C. Souberbielle, C. Massart, S. Brailly-Tabard, E. Cavalier, P. Chanson, Prevalence and determinants of vitamin D deficiency in healthy French adults: the VARIETE study, Endocrine. 53 (2016) 543–550. https://doi.org/10.1007/s12020-016-0960-3.

[39] NDNS: results from Years 5 and 6 (combined), GOV.UK. (n.d.). https://www.gov.uk/government/statistics/ndns-results-from-years-5-and-6-combined (accessed April 26, 2020).

[40] Bates B, Lennox A, Prentice A, Bates C, Page P, Nicholson S, Swan G., National Diet and Nutrition Survey: headline results from years 1 to 4 (combined) of the Rolling Programme for 2008 and 2009 to 2011 and 2012. 2014., (n.d.).

[41] Z. Wu, J.M. McGoogan, Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention, JAMA. (2020). https://doi.org/10.1001/jama.2020.2648.

[42] KCDC, KCDC, KCDC. (n.d.). http://www.cdc.go.kr (accessed April 3, 2020).

[43] covid-19-point-epidemiologique-du-15-mars-2020, (n.d.). https://www.santepubliquefrance.fr/maladies-et-traumatismes/maladies-et-infections-respiratoires/infection-a-coronavirus/documents/bulletin-national/covid-19-point-epidemiologique-du-15-mars-2020 (accessed March 19, 2020).

[44] RKI - Startseite, (n.d.). https://www.rki.de/DE/Home/homepage_node.html (accessed April 6, 2020).

[45] A.K. Simon, G.A. Hollander, A. McMichael, Evolution of the immune system in humans from infancy to old age, Proc. R. Soc. B Biol. Sci. 282 (2015). https://doi.org/10.1098/rspb.2014.3085.

[46] S. Mahbub, A.L. Brubaker, E.J. Kovacs, Aging of the Innate Immune System: An Update, Curr. Immunol. Rev. 7 (2011) 104–115. https://doi.org/10.2174/157339511794474181.

[47] C. Qin, L. Zhou, Z. Hu, S. Zhang, S. Yang, Y. Tao, C. Xie, K. Ma, K. Shang, W. Wang, D.-S. Tian, Dysregulation of Immune Response in Patients with COVID-19 in Wuhan, China, Social Science Research Network, Rochester, NY, 2020. https://papers.ssrn.com/abstract=3541136 (accessed April 4, 2020).

[48] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, Innate Immunity, Mol. Biol. Cell 4th Ed. (2002). https://www.ncbi.nlm.nih.gov/books/NBK26846/ (accessed April 3, 2020).

[49] X. Chen, S. Liu, M.U. Goraya, M. Maarouf, S. Huang, J.-L. Chen, Host Immune Response to Influenza A Virus Infection, Front. Immunol. 9 (2018). https://doi.org/10.3389/fimmu.2018.00320.

[50] Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, The Lancet. 395 (2020) 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.

[51] P. Little, Non-steroidal anti-inflammatory drugs and covid-19, BMJ. 368 (2020). https://doi.org/10.1136/bmj.m1185.

[52] R. Mortensen, H.S. Clemmensen, J.S. Woodworth, M.L. Therkelsen, T. Mustafa, K. Tonby, S. Jenum, E.M. Agger, A.M. Dyrhol-Riise, P. Andersen, Cyclooxygenase inhibitors impair CD4 T cell immunity and exacerbate Mycobacterium tuberculosis infection in aerosol-challenged mice, Commun. Biol. 2 (2019) 1–10. https://doi.org/10.1038/s42003-019-0530-3.

[53] S. Bancos, M.P. Bernard, D.J. Topham, R.P. Phipps, Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells, Cell. Immunol. 258 (2009) 18–28. https://doi.org/10.1016/j.cellimm.2009.03.007.

[54] Y.-J. Lee, Y.-C. Chuang, Ibuprofen augments pro-inflammatory cytokine release in a mouse model of Vibrio vulnificus infection, Microbiol. Immunol. 54 (2010) 542–550. https://doi.org/10.1111/j.1348-0421.2010.00249.x.

[55] L. Sirota, D. Shacham, I. Punsky, H. Bessler, Ibuprofen affects pro- and anti-inflammatory cytokine production by mononuclear cells of preterm newborns, Biol. Neonate. 79 (2001) 103–108. https://doi.org/10.1159/000047075.