

Vitamin D Status and COVID-19: Some Implications

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Abstract

Background Vitamin D is essential for the maintenance of good health and its status is defined by the level of serum 25-hydroxyvitamin (25(OH)D). Negative correlations between mean levels of 25(OH)D per country and the number of COVID-19 confirmed cases per one million population, and COVID-19 mortality per one million population, were recently observed. The aim of this study was to identify levels of 25(OH)D below which, rates of COVID-19 confirmed cases, mortality and lethality, increase significantly.

Methods A data table found in the literature, containing a list of twenty countries and their corresponding mean level of 25(OH)D was updated with COVID-19 latest numbers of confirmed cases and mortality rates. Cut points of 25(OH)D below which rates were significantly higher were found according to various statistical criteria: absolute difference of means, *t*-test *p*-values, between class variance, entropy. Thresholds of 25(OH)D below which there can be a significant rise of COVID-19 confirmed cases, mortality and lethality, were found performing a Gaussian kernel regression.

Results All the criteria yielded 25(OH)D cut off points at 50 ± 10 nmol/L for Cases and Mortality variables and at 60 ± 10 nmol/L for Lethality variable. A Gaussian kernel regression showed a significant rise in the rates of COVID-19 confirmed cases, mortality and lethality, below 25(OH)D levels of 60 ± 6 nmol/L.

Conclusion In this study, our method provided a reliable estimate (95 % confidence interval) of 25(OH)D levels, of 60 ± 6 nmol/L, in the range of vitamin D insufficiency, below which rates of COVID-19 confirmed cases per one million population, rates of COVID-19 mortality per one million population increased. We found that 25(OH)D levels below 50 ± 10 nmol/L, mainly in the range of vitamin D deficiency, associate with highest COVID-19 mortality rates. Therefore, we suggest that 25(OH)D concentrations should be above 60 ± 6 nmol/L to reduce morbidity and mortality during the COVID-19 pandemic.

Background

Vitamin D is essential for the maintenance of good health. There are two ways to get vitamin D: either orally from the diet and vitamin D supplements or dermally, through synthesis in the skin, in response to solar ultraviolet B radiation. In humans, dermal production is the major source of vitamin D. Vitamin D is hydroxylated in the liver into 25-hydroxyvitamin [25(OH)D] and subsequently in the kidney to 1,25-dihydroxy vitamin D [1,25(OH)₂D]. This is the active metabolite, which stimulates intestinal calcium absorption. Vitamin D status is defined by serum 25(OH)D concentration [1, 2], which is influenced in turn by various factors such as latitude and skin pigmentation [3].

The production of 1,25(OH)₂D is stimulated by parathyroid hormone, or parathormone (PTH). There is a negative feedback through calcium, which decreases PTH, and a direct negative feedback from 1,25(OH)₂D to PTH. PTH increases the reabsorption of calcium in the kidney and stimulates bone resorption, resulting in an increase in serum calcium. When 25(OH)D availability declines, serum 1,25(OH)₂D declines, and this results in reduced calcium absorption, a transient decline in serum calcium concentration, and stimulation of PTH secretion known as secondary hyperparathyroidism.

The recent outbreak and rapid spreading of severe acute respiratory syndrome COVID-19 are a global threat and primary concern worldwide, with a still uncertain outcome. Previous observational studies report independent associations between low serum concentration of 25-hydroxyvitamin D and susceptibility to acute respiratory tract infections [4]. Some authors found that vitamin D might play a protective role for COVID-19 with an association between the mean levels of vitamin D in various countries and the morbidity, and the mortality, caused by COVID-19 [5].

Our purpose was to estimate 25(OH)D levels below which rates of COVID-19 confirmed cases per one million population (/1 M), rates of COVID-19 mortality/1 M, and lethality, were higher.

Methods

We used the aggregated data table built by Ilie et al. in [5] with the same sample of $n = 20$ countries and their corresponding mean level of 25(OH)D, but the number of COVID-19 confirmed cases/1 M and the number of COVID-19 deaths/1 M population were updated according to the recent records of Coronavirus Disease 2019 (COVID-19) data in the WHO records [6]. Lethality variable, ratio of the number of deaths/1 M to the number of confirmed cases/1 M, was computed. It was an estimation of the probability of dying knowing that the patient is infected.

K -means algorithm was applied with $K = 2$ to Cases variable in order to get a partition of two clusters Δ and ∇ composed of countries having a higher number of cases/1 M and a lower number of cases/1 M, respectively.

In order to find a 25(OH)D cut point below which the behavior of Cases variable statistically differs significantly from the behavior of Cases variable above that cut point, the table was first ordered in increasing order of 25(OH)D (Table 1) and spitted into a partition of two classes:

- - Class 1: countries such that $25(\text{OH})\text{D} \leq c$
- - Class 2: countries such that $25(\text{OH})\text{D} > c$

where c denotes a 25(OH)D concentration level varying through the range of country 25(OH)D levels.

For $i = 1, 2$, let n_i be Class i size and let m_i be the mean of Cases variable in Class i .

The best choice of c depended on various possible criteria:

- - c maximizes $|m_1 - m_2|$
- - c minimizes the p -value in a student t -test comparing m_1 and m_2 , the null and the alternative hypotheses being $H_0 : m_1 = m_2$ and $H_1 : m_1 > m_2$, respectively
- - c maximizes the Between Class Variance (BCV) defined as follows:

$$\text{BCV} = (n_1 (m_1 - m)^2 + n_2 (m_2 - m)^2) / n.$$

BCV indicates how Class 1 and Class 2 differ from the whole sample and, when it is maximal, the Within Class Variance is minimal. BCV is a standard evaluation of the classification quality.

- c minimizes the entropy H of Class 1 - Class 2 partition defined as follows:

\cap denoting intersection and $||$ cardinality, for $i = 1, 2$, Class i entropy H_i is defined as

$$H_i = -r \log_2(r) - (1-r) \log_2(1-r) \text{ where } r = |\text{Class } i \cap \Delta| / n_i \text{ and } H = (n_1 H_1 + n_2 H_2) / n.$$

Statistical procedures were implemented using R 3.6.3 software.

Confidence Intervals of 95% were adjusted bootstrap percentile intervals obtained using the 'boot.ci' function, option type = 'bca', in the 'boot' package. They were presented in the form *original* [*le*, *re*], where *original* denoted the estimation from the original sample Table 1, *le* and *re* denoting the confidence interval (CI) left and right endpoints, respectively. The number of bootstrapped samples of size $n = 20$, was $R = 1,000$. Actually, the value *original* was not the midpoint of the CI [*le*, *re*].

Linear regressions were applied to our sample, confidence intervals and p -values were derived from Dupont-Plummer formulas [7]. The rise of Cases variable was investigated by performing a Gaussian kernel regression of Cases variable on 25(OH)D concentrations using the 'npreg' function in the 'np' (nonparametric) package.

The preceding methods were applied similarly to Mortality and Lethality variables, respectively.

Results

The results presented below in Table 1, were obtained using the COVID-19 data recorded on 2020 July 27th [6]. No significant differences were observed in the results when using COVID-19 data recorded two weeks before or two weeks after that date because infection and mortality rates per one million population did not vary significantly.

Table 1 Mean level of 25(OH)D in increasing order, cases of COVID-19/1 M, deaths caused by COVID-19/1 M, lethality (27th July 2020)

Countries	25(OH)D means (nmol/L)	Cases/1 M	Deaths/1 M	Lethality
Portugal	39	4934	169	0.03
Spain	42.5	6969	608	0.09
Switzerland	46	3997	228	0.06
Denmark	47	2338	106	0.05
UK	47.4	4419	674	0.15
Belgium	49.3	5730	847	0.15
Italy	50	4074	581	0.14
Germany	50.1	2475	110	0.04
Estonia	51	1536	52	0.03
Turkey	51.8	2690	67	0.02
Ireland	56.4	5239	357	0.07
Island	57	5431	29	0.01
Netherlands	59.5	3101	358	0.12
France	60	2804	463	0.17
Hungary	60.6	461	62	0.13
Czechia	62.5	1449	35	0.02
Norway	63.5	1684	47	0.03
Finland	67.7	1336	59	0.04
Sweden	73.5	7858	564	0.07
Slovakia	81.5	404	5	0.01

The results for the number of cases, for mortality and for lethality were summarized in Table 2, Table 3 and Table 4, respectively.

K-means algorithm applied with $k = 2$ for example to Mortality variable, using the original Table 1 sample, yielded two clusters of countries, one cluster of 8 countries (Spain, United Kingdom, Belgium, Italy, Ireland, The Netherlands, France, Sweden) having an elevated mortality rate and a second cluster of 12 countries (Portugal, Switzerland, Denmark, Germany, Estonia, Turkey, Island, Hungary, Czechia, Norway, Finland, Slovakia) having a moderated or low mortality rate, see Table 3.

Table 2 Statistics for the Number of Cases/1 M

Methods	Cut points c (nmol/L)	Statistics for Cases/1 M
Sample size		$n = 20$
Mean		$m = 3446$ [2654 4403]
Standard Deviation		$s = 2112$ [1634 2699]
Maximum		7,858 (Sweden)
Minimum		404 (Slovakia)
Correlation to 25(OH)D		- 0.338 ($p = 0.073$)
K -means algorithm ($K = 2$) Δ : cluster of countries having a higher number of Cases/1 M \square : cluster of countries having a lower number of Cases/1 M		$\Delta = \{\text{Portugal, Spain, Switzerland, UK, Belgium, Italy, Ireland Island, Sweden}\}.$ Cases/1 M > 3200 $\square = \{\text{Denmark, Germany, Estonia, Turkey, Netherland, France, Hungary, Czechia, Norway, Finland, Slovakia}\}.$ Cases/1 M ≤ 3200
Maximal Between Class Absolute Difference of means	49.3 [40 60]	$m_1 = 4731.17, m_2 = 2895.86$ $m_1 - m_2 = 1835.61$ $m_1 > m_2 (p = 0.026)$ at $c = 49.3$
Minimal t -test p -value	50 [43 67]	$p = 0.019$ at $c = 50$
Maximal Between Class Variance	50 [42.5 59.5]	$BCV = 765,000$ at $c = 50$
Minimal Entropy	50 [42.5 57]	$H = 0.7137$ at $c = 50$
Gaussian Kernel Regression		Rise starting at 61 ± 6 nmol/L

Class 1: Countries such that $25(\text{OH})\text{D} \leq c$

Class 2: Countries such that $25(\text{OH})\text{D} > c$

Table 3 Statistics for Mortality/1 M

Methods	Cut points c ($nmol/L$)	Statistics for Mortality/1 M
Sample size		$n = 20$
Mean		$m = 271$ [171 396]
Standard Deviation		$s = 263$ [213 338]
Maximum		847 (Belgium)
Minimum		5 (Slovakia)
Correlation with 25(OH)D		- 0.276 ($p = 0.119$)
K -means algorithm ($K = 2$) Δ : cluster of countries having a higher mortality/1 M \square : cluster of countries having lower mortality/1 M		$\Delta = \{\text{Spain, UK, Belgium, Italy, Ireland, Netherland, France, Sweden}\}.$ Deaths/1 M > 230 $\square = \{\text{Portugal, Switzerland, Denmark, Germany, Estonia, Turkey, Island, Hungary, Czechia, Norway, Finland, Slovakia}\}.$ Deaths/1 M \leq 230
Maximal Between Class Absolute Difference of means	50 [43 59]	$m_1 = 459.88, m_2 = 169.85$ $m_1 - m_2 = 290.03$ $m_1 > m_2 (p = 0.020)$ at $c = 50$
Minimal t -test p -value	50 [43 56]	$p = 0.020$ at $c = 50$
Maximal Between Class Variance	50 [39 56]	$BCV = 19,021$ at $c = 50$
Minimal Entropy	60 [48 72]	$H = 0.8950$ at $c = 50$
Gaussian Kernel Regression		Rise starting at $62s \pm 6 nmol/L$

Class 1: Countries such that $25(OH)D \leq c$

Class 2: Countries such that $25(OH)D > c$

Table 4: Statistics for Lethality variable

Methods	Cut points c (nmol/L)	Statistics for Lethality
Sample size $n = 20$		$n = 20$
Mean		$m = 0.072$ [0.050 0.097]
Standard Deviation		$s = 0.053$ [0.042 0.063]
Maximum		0.17 (France)
Minimum		0.01 (Slovakia)
Correlation with 25(OH)D		- 0.195 ($p = 0.205$)
K -means algorithm ($K = 2$)		$\Delta = \{\text{Spain, UK, Belgium, Italy, Netherland, France, Hungary}\}.$
Δ : cluster of countries having higher lethality		Lethality > 0.08
\square : cluster of countries having lower lethality		$\square = \{\text{Portugal, Switzerland, Denmark, Germany, Estonia, Turkey, Ireland, Island, Czechia, Norway, Finland, Sweden, Slovakia}\}.$
		Lethality < 0.08
Maximal Between Class Absolute Difference of means	60.6 [49 73]	$m_1 = 0.084, m_2 = 0.034$ $m_1 - m_2 = 0.05$ $m_1 > m_2 (p < 0.01)$ at $c = 60.6$
Minimal t -test p -value	60.6 [49 73]	$p = 0.0055$ at $c = 60.6$
Maximal Between Class Variance	60.6 [49 73]	$BCV = 0.0005$ at $c = 60.6$
Minimal Entropy	60.6 [48 67]	$H = 0.7476$ at $c = 60.6$
Gaussian Kernel Regression		Rise starting at 63 ± 6 nmol/L

Class 1: Countries such that $25(\text{OH})\text{D} \leq c$

Class 2: Countries such that $25(\text{OH})\text{D} > c$

For the Cases variable, the cut point c was found around 50 nmol/L for any of the four criteria from Table 1 sample, see Fig. 1 and Fig. 2. The `boot.ci` function performed the procedure on each of the $R = 1,000$ bootstrapped samples to get a 95% CI displayed in Table 2.

Linear regressions applied to our data were not significant, confidence intervals were not acute enough and p -values derived from Dupont-Plummer formulas [7] were bad, even using bootstrap methods. The nonparametric Gaussian Kernel regression curve of the morbidity variable on 25(OH)D, showed a rising starting at 61 nmol/L when using Table 1 data, see Fig. 3. That of mortality variable showed a rising at 62 nmol/L when using Table 1 data, see Fig. 4.

In our Table 1 sample, we noticed that there was only one country, namely Sweden, over the 6 countries having 25(OH)D mean strictly greater than 60nmol/L, which had an elevated mortality rate, while 7 countries over the 14 having 25(OH)D less than 60 nmol/L, had an elevated mortality rate, see Fig. 4.

However, 25(OH)D is certainly not the only factor that could predict mortality clusters \square and \square : we observed that the AUC (Area Under Curve) of ROC curves were lower than 0.65 when performing the *prediction* function in the ROCR package of *R* software.

Discussion

The general metabolism and actions of vitamin D are well-known, in particular as a natural immune modulator [8]. Vitamin D enhances cellular innate immunity partly through the induction of antimicrobial peptides [9, 10, 11]. It also reduces the cytokine storm induced by the innate immune system in response to viral and bacterial infections, as it was observed in COVID-19 patients [12].

In the literature, we found several thresholds of 25(OH)D concentrations which defined vitamin D deficiency. The institute of Medicine defined vitamin D deficiency threshold at 25(OH)D level of 30 nmol/L [13]. Vitamin D deficiency is defined at 100 nmol/L for the vitamin D Council [14], and at 50 nmol/L for the Endocrine society [15]. Our findings, which seems to be consistent with the Endocrine Society estimate, suggested that a 25(OH)D levels below 50 ± 10 nmol/L may be linked to severe clinical outcomes of COVID-19 infection. Thus, maintaining a serum 25(OH)D levels above 50 nmol/L could be recommended in COVID-19 disease. Indeed, Casey et al. [16] concluded that daily supplementation of vitamin D potentially offer additional protection against COVID-19 and Ebadi et al. [17] noticed that a high-dose vitamin D intervention could have a potential benefit in decreasing risk of COVID-19 severity and mortality.

Many investigators have shown that there is a threshold for serum 25(OH)D below which secondary hyperparathyroidism may occur [18] and this threshold has been estimated in the region of 40–50 nmol/L [19, 20, 21]. As we found that COVID-19 mortality rates below vitamin D level of 50 ± 10 nmol/L are statistically higher than the rates above 50 nmol/L, it could be assumed that elevation of

PTH concentrations are involved in COVID outcomes on most of patients who have a 25(OH)D level below 50 nmol/L. Indeed, some studies have shown that elevated PTH levels are associated with increased cardiovascular risk in the general population [22, 23]. Mitnick et al. [24] have found that the liver is an important source of the circulating interleukin-6 generated in response to PTH while Casey et al. [16] have indicated that the most severe cases in COVID involving a pro-inflammatory state can lead to harmful outcomes mediated by a deregulated immune response involving interleukin-6 and other inflammatory signaling molecules. Moreover, Cheng et al. [25] have found an association between higher serum concentrations of PTH and several inflammatory markers.

It is known that 1,25(OH)₂D concentrations depend mainly of 25(OH)D levels. According to White et al. [26], the influence of 1,25(OH)₂D on the immune system is one of the most important factors to consider. Notably, 1,25(OH)₂D regulates the immune system via the vitamin D receptor which is present on most of the immune cell types, particularly in antigen-presenting cells such as monocytes, macrophages and dendritic cells. According to Mangin et al. [27], circulating levels of 25(OH)D may not be an accurate reflection of vitamin D status because among patients with systemic inflammatory diseases, 1,25(OH)₂D might be elevated so that low serum 25(OH)D, despite adequate photosynthesis of vitamin D, could be a consequence of an inflammatory process. The authors indicated that measuring both 25(OH)D and 1,25(OH)₂D provide a true picture of vitamin D status rather than measuring 25(OH)D alone. Therefore, measuring 1,25(OH)₂D as a marker of inflammation in each stage of clinical evolution of COVID-19 patients could be in one's interest.

Concerning the role of calcium concentration in COVID-19 mortality, Lippi et al. [28] found that COVID-19 pandemic severity is in part associated with lower serum concentrations of calcium. It is well-known that the ionic concentration of calcium, which is highly correlated to 25(OH)D and to 1,25(OH)₂D, appears as the major factor influencing the secretion rate of PTH [29]. PTH plays a critical role in calcium homeostasis, defending against hypocalcemia by acting on target organs such bone and kidney in order to stimulate bone resorption, to promote renal conservation of calcium, and to induce production of 1,25(OH)₂D, which in turn enhances intestinal calcium absorption [30]. Moreover, Goodman et al. [31] noticed that a slight decrease in ionized calcium triggers the release of PTH from the parathyroid glands, suggesting that patients with severe COVID-19 and low calcium concentrations may have high levels of PTH that could worsening COVID-19 issues. More studies are needed to investigate this suggestion.

In the past, vitamin D deficiency was identified by the presence of bone diseases, essentially either rickets or osteomalacia. More recently, the term vitamin D insufficiency has been used to describe suboptimal levels of serum 25(OH)D that might be associated with other disease outcomes [32]. According to Holick [33], Vitamin D insufficiency is now recognized as 25(OH)D concentrations of 52–72 nmol/L. In our study we found a 25(OH)D threshold of 60 ± 6 nmol/L below which there is a significant rise in the number of COVID-19 cases and mortality and lethality. Such a threshold is in the range of vitamin D insufficiency. It could be suggested that patients with vitamin D insufficiency might have harmful outcomes with COVID-19 before reaching status of vitamin D deficiency. Mc Kenna et al. found that when serum 25(OH)D concentrations are in the range of vitamin D insufficiency, PTH levels can be slightly elevated but are still

in the normal range [34]. This was confirmed in the results presented in Emilion et al. study [20]: the regression curve of PTH concentrations on 25(OH)D concentrations showed a small rise of PTH levels at 59 nmol/L, a plateau in the range 49–59 nmol/L and a main rise at 49 nmol/L. Moreover, when patient becomes vitamin D insufficient and deficient, the increase in PTH levels result in normal or elevated levels of 1,25(OH)2D [34]. Then, 1,25(OH)2D assay could be useless as a measure of vitamin D status [33].

In this study, we were not informed of PTH levels, 1,25(OH)2D levels, and calcium concentrations of the patients with COVID-19 from countries. Though the vitamin D cut-points of 50 ± 10 nmol/L should not be interpreted as an optimal vitamin D status, it could be used in the monitoring of vitamin D supplementations in COVID-19 syndrome. Future works need at least to take in account, for patients with COVID-19 from different stages of clinical evolution, measurements of concentrations of 25(OH)D, 1,25(OH)2D, calcium and PTH in order to elaborate reliable protocols of vitamin D-calcium supplementations and to realize a more efficient clinical monitoring of COVID-19 infection.

Conclusion

We identified 25(OH)D levels, of 60 ± 6 nmol/L, in the range of vitamin D insufficiency, below which rates of COVID-19 confirmed cases per one million population, and rates of COVID-19 mortality per one million population increased. We found that below 25(OH)D levels, of 50 ± 10 nmol/L, mainly in the range of vitamin D deficiency, rates of COVID-19 mortality were the highest. Therefore, we suggest that 25(OH)D concentrations should be above 60 ± 6 nmol/L to reduce morbidity and mortality during the COVID-19 pandemic. Future works could investigate other potential roles of vitamin D status in the evolution of the COVID-19 syndrome.

Abbreviations

COVID-19 = Corona Virus Disease 2019. **/1 M** = per 1 million population, **nmol/L** = nano moles per liter, **BCV** = Between Class Variance, **CI** = Confidence Interval

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Competing Interest

Not applicable

Funding

Not applicable

Authors' contributions

EE initiated the medical problem and wrote the Abstract, the Background, the Discussion and the Conclusion sections. RE analyzed the data, proposed the statistical methods, implemented the statistical procedures, and wrote the Methods and Results parts. All authors read and approved the final manuscript.

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EE obtained is PhD in medicine from University of Paris VI. He is a medicine Doctor at Community Clinic, Paris, France. RE obtained is PhD in pure mathematics from University Paris VI. He is an emeritus professor of applied mathematics at Denis Poisson Institute, University of Orléans, France.

Footnotes

Not applicable

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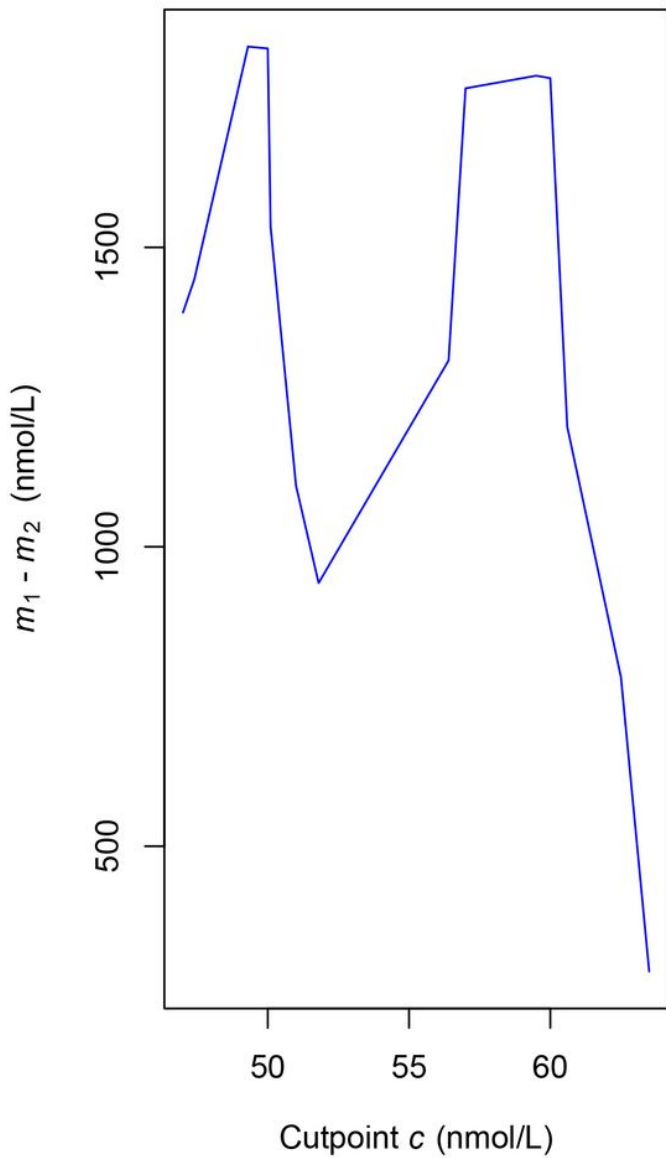
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Figures

Between Class Difference of Means



t-test p-values

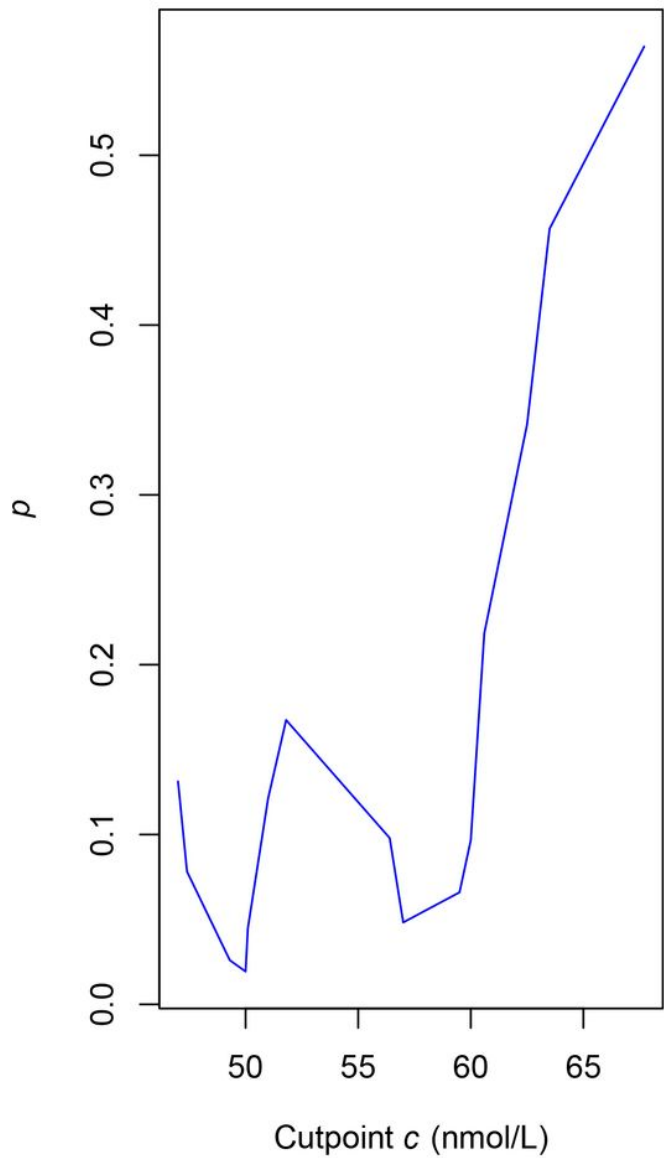
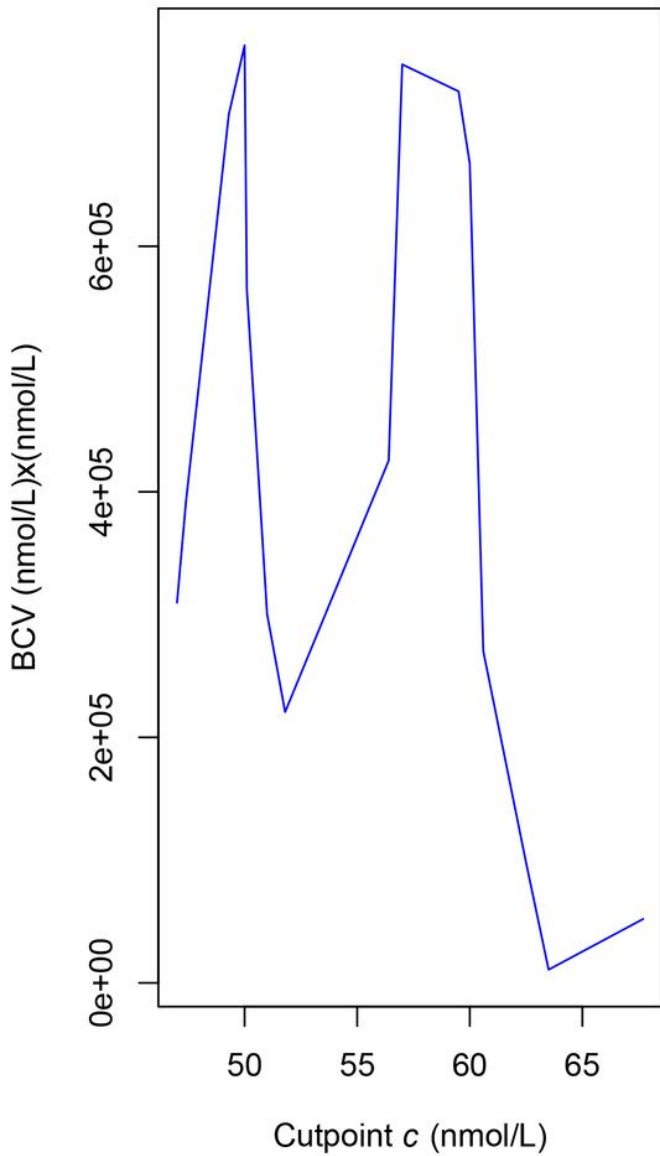


Figure 1

Morbidity variable: Cut point for Difference of means and p-values criteria Cut point c varying through the range of 25(OH)D in Table 1 sample Class 1: Countries such that $25(OH)D \leq c$. Mean of Cases in Class 1 = m_1 Class 2: Countries such that $25(OH)D > c$. Mean of Cases in Class 2 = m_2 $|m_1 - m_2|$ was maximal at $c = 49.3$ nmol/L (left panel) t-test p-value was minimal at $c = 50$ nmol/L (right panel)

Between Class Variance (BCV)



Entropy of the Partition

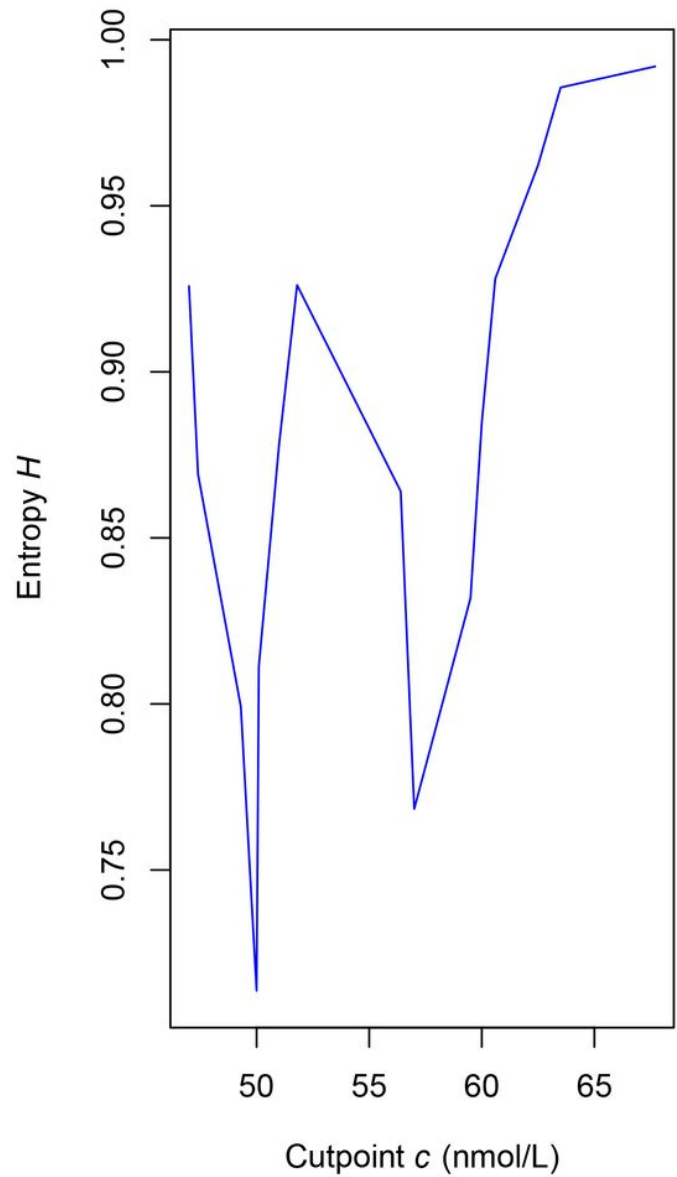


Figure 2

Morbidity variable: Cut point for BCV and Entropy criteria Cut point c varying through the range of 25(OH)D in Table 1 sample Class 1: Countries such that $25(OH)D \leq c$. Mean of Cases in Class 1 = m_1 Class 2: Countries such that $25(OH)D > c$. Mean of Cases in Class 2 = m_2 Between Class Variance (BCV) was maximal at $c = 50$ nmol/L (left panel) Partition Entropy H was minimal at $c = 50$ nmol/L (right panel)

Fig. 3

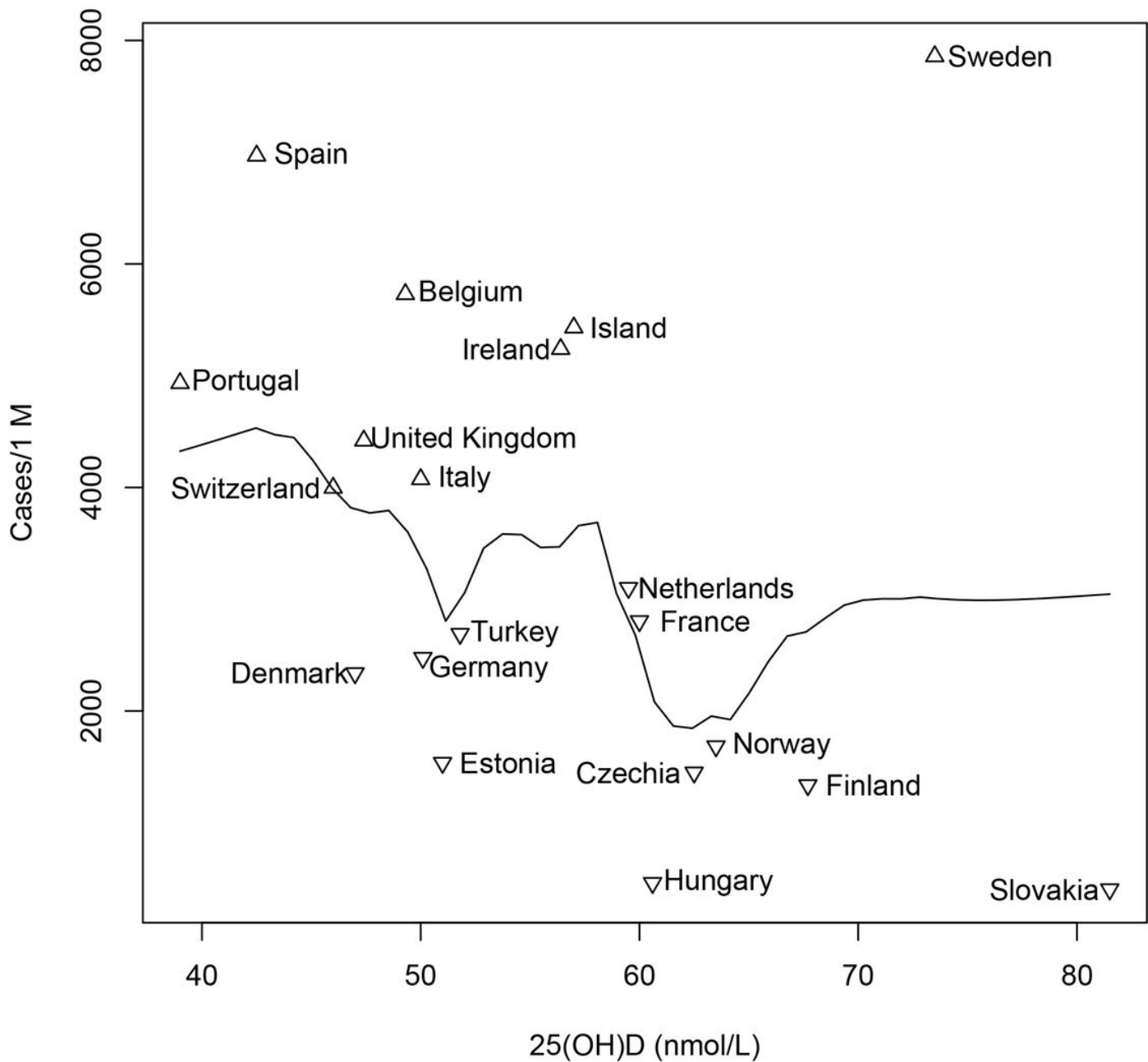


Figure 3

Gaussian Kernel regression of morbidity variable on 25(OH)D. Rising started at 61 nmol/L. K-means algorithm applied to mortality variable yielded a cluster of countries having a high morbidity rate (point up triangle \triangle) and a cluster of countries having a low morbidity rate (point down triangle ∇). The regression curve separated the two clusters.

Fig. 4

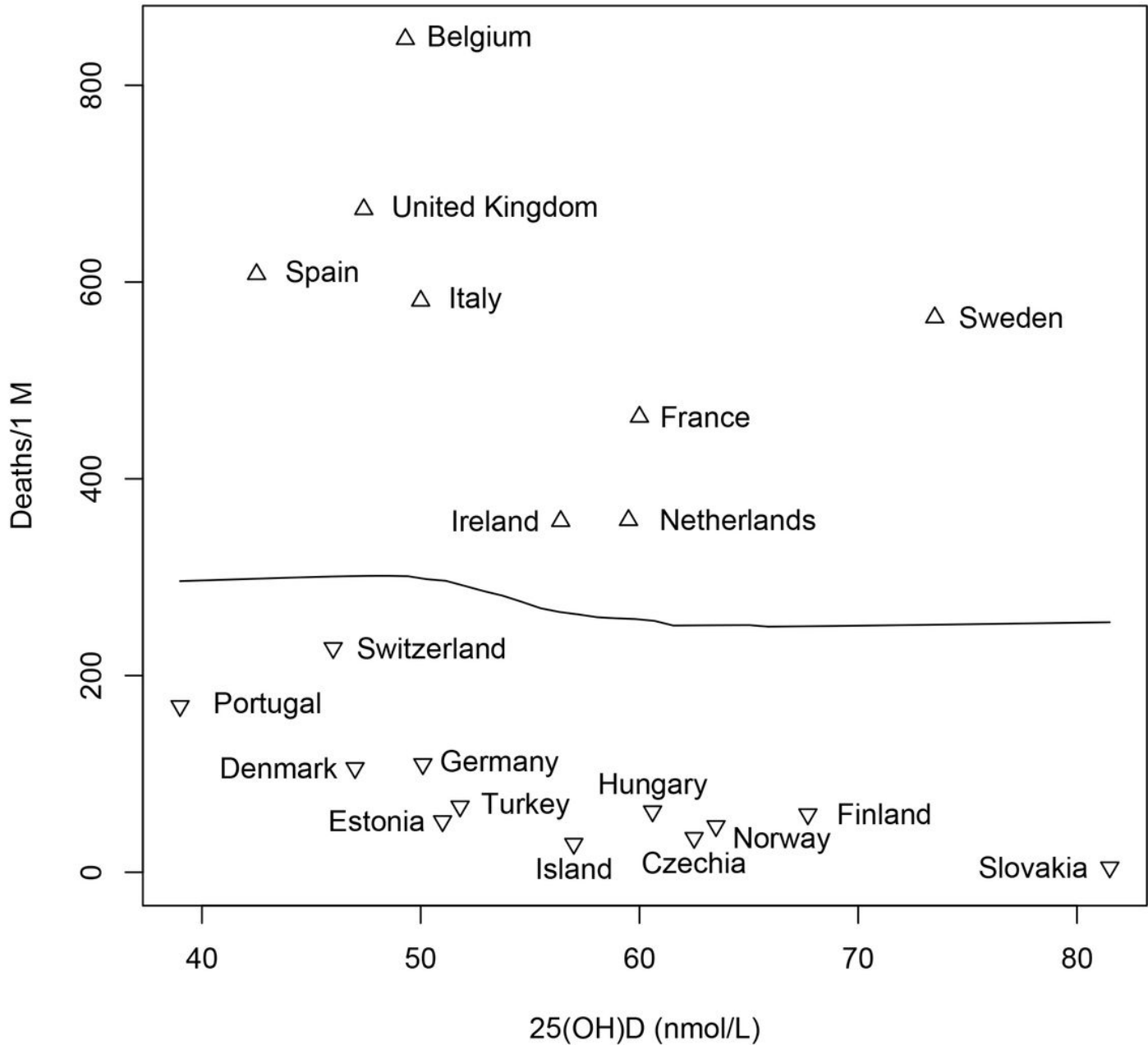


Figure 4

Gaussian Kernel regression of mortality variable on 25(OH)D. Rising started at 62 nmol/L. K-means algorithm applied to mortality variable yielded a cluster of countries having a high morbidity rate (point up triangle \triangle) and a cluster of countries having a low morbidity rate (point down triangle ∇). The regression curve separated the two clusters.