Vitamin D deficiency is associated with higher hospitalisation risk from COVID-19: a retrospective case-control study

Edward B Jude MD[1,2,3], Stephanie F Ling MRCP[1,2], Rebecca Allcock FRCPath[4], Beverly XY Yeap MBChB[2], Joseph M Pappachan FRCP[2,3,4]

1. Tameside and Glossop Integrated Care NHS Foundation Trust, Fountain Street, Ashton-under-Lyne, OL6 9RW, UK.
2. The University of Manchester, Oxford Road, Manchester, M13 9PL, UK.
3. Manchester Metropolitan University, All Saints Building, Manchester, M15 6BH, UK.
4. Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust, Sharoe Green Lane, Fulwood, Preston, PR2 9HT, UK.

Corresponding author:

Professor Edward Jude, Tameside and Glossop Integrated Care NHS Foundation Trust

Fountain Street, Ashton-under-Lyne, OL6 9RW, UK; Edward.jude@tgh.nhs.uk

Telephone: +44 (0) 161 922 5189

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Abstract

CONTEXT: One of the risk factors for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is postulated to be vitamin D deficiency. To understand better the role of vitamin D deficiency in the disease course of COVID-19, we undertook a retrospective case-control study in the North West of England (NWE).

OBJECTIVE: To examine whether hospitalisation with COVID-19 is more prevalent in individuals with lower vitamin D levels.

METHODS: The study included individuals with results of serum 25-hydroxyvitamin D (25[OH]D) between 1 st April 2020 and 29th January 2021. Patients were recruited from two districts in NWE. The last 25(OH)D level in the previous 12 months was categorised as 'deficient' if less than 25 nmol/L and 'insufficient' if 25-50 nmol/L.

RESULTS: 80,670 participants were entered into the study. Of these, 1,808 were admitted to hospital with COVID-19, of whom 670 died. In a primary cohort, median serum 25(OH)D in participants who were not hospitalised with COVID-19 was 50.0 [interquartile range, IQR 34.0-66.7] nmol/L versus 35.0 [IQR 21.0-57.0] nmol/L in those admitted with COVID-19 ($p < 0.005$). There were similar findings in a validation cohort (median serum 25(OH)D 47.1 [IQR 31.8-64.7] nmol/L in non-hospitalised versus 33.0 [IQR 19.4-54.1] nmol/L in hospitalised patients). Age-, sex- and seasonal variation-adjusted odds ratios for hospital admission were 2.3-2.4 times higher among participants with serum 25(OH)D <50 nmol/L, compared to those with normal serum 25(OH)D levels, without any excess mortality risk.

CONCLUSIONS: Vitamin D deficiency is associated with higher risk of COVID-19 hospitalisation. Widespread measurement of serum 25(OH)D and treating any unmasked insufficiency or deficiency through testing may reduce this risk.

Key words: Vitamin D deficiency; COVID-19; Hospitalisation; Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

INTRODUCTION:

Vitamin D deficiency has been proposed as a risk factor for many viral respiratory illnesses, including SARS-CoV-2 infection. Despite attempts to curtail coronavirus disease-2019 (COVID-19) resulting from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, there has been only limited success, even after 18 months since the beginning of the mega-pandemic in December 2019.

Vitamin D has been thought to possess immunomodulatory functions, apart from its well-known effects on bone mineral metabolism.

Several observational studies[1-10] examined the role of vitamin D on COVID-19 infection risk or disease outcomes with conflicting results. Although with some technical flaws and discrepancies in the assessment methods, three systematic reviews also assessed the risk of COVID-19 and vitamin D levels, and the benefits of supplementation on morbidity and mortality reduction from the disease.[11-13] These reviews suggested an overall increased risk of hospitalisation (odds ratios [OR] 1.43-1.81) and mortality (OR 1.82, 95% confidence interval [CI] 1.6-2.58) from COVID-19 in patients with vitamin D deficiency. However, the studies included in these systematic reviews exhibited a high risk of various biases such as inadequate evaluation of the outcome, inappropriate sample selection and lack of uniformity of the inclusion criteria, and the certainty of evidence emerging from these studies appears low. On the contrary, a study from Italy[14] and another one from Brazil[15] clearly refute the probability of a causal link between vitamin D deficiency and susceptibility to SARS-CoV-2 infection. Therefore, it is imperative to have more evidence based on large population-based studies to reveal the risk of COVID-19 in populations with vitamin D deficiency, and multi-centre randomised controlled trials (RCTs) to observe the potential benefits of vitamin D supplementation in treating the disease.

Most studies that have looked at prevailing vitamin D levels and COVID-19 have been performed in a small number of patients. Therefore, we set out to understand the role of vitamin D levels and the risk of developing COVID-19 in a large-cohort observational study from two hospital sites in the North West of England (NWE) in the United Kingdom (UK) to uncover the uncertainties around this highly debated topic. Our primary outcome was to determine whether insufficient or deficient vitamin D status was associated with increased risk of hospitalisation from COVID-19, with due consideration of Northern hemisphere seasonal variations of vitamin D. Our secondary outcome was to determine whether insufficient or deficient vitamin D status was associated with increased risk of in-patient death from COVID-19.

METHODS

Participants

Participants were recruited to a primary cohort if they had a serum 25-hydroxyvitamin D (25[OH]D) level carried out at the hospital laboratory based at Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust, between 1st April 2019 and 29th January 2021. Participants were recruited to a validation cohort if they had a serum 25(OH)D level carried out at the hospital laboratory based at Tameside General Hospital, Tameside and Glossop Integrated Care NHS Foundation Trust, between 1st April 2019 and 29th January 2021. Cases were identified from the biochemistry database of both the recruitment sites by an electronic search for 25(OH)D performed during the above period. COVID-19 patients were also identified from the inpatient electronic admission register. Laboratory testing for COVID-19 was carried out using throat ±nasal swab, and samples were tested for SARS-CoV-2 viral RNA following amplification using real-time PCR. Patients

were included if they were aged 18 years or older. Serum 25(OH)D measured for all indications were included, although information on indications was not available. 25(OH)D measurements were carried out for a mixture of in-patients, outpatients and primary care. Vitamin D insufficiency was defined as serum 25(OH)D levels 25-50 nmol/L, and deficiency as <25 nmol/L as per the current National Institute for Health and Clinical Care Excellence (NICE), UK guidelines.[16] A low vitamin D category was created by combining both vitamin D insufficient and deficient patients. Participants were recruited both from the community and in-patient stays. In-patients who had been admitted to hospital with COVID-19 with serum 25(OH)D samples measured more than 365 days prior to eventual discharge date were excluded. Furthermore, patients with serum 25(OH)D samples carried out after discharge were also excluded, as were COVID-19 in-patients who did not have 25(OH)D measured during the study period.

Serum 25(OH)D measurement

Serum 25(OH)D was measured using the cobas e 801 analytical unit (Roche, Basel, Switzerland) at Royal Preston Hospital, and using the UniCel Dxl 800 Access Immunoassay System (Beckman Coulter Life Sciences, Indianapolis, USA) at Tameside General Hospital. The clinical laboratories at Royal Preston Hospital participate in the Vitamin D External Quality Assessment Scheme (DEQAS)[17] to ensure analytical reliability of its 25(OH)D assays. The clinical laboratories at Tameside General Hospital participate in the Randox International Quality Assessment Scheme (RIQAS)[18] in order to ensure external quality of all assays, including 25(OH)D. Because serum 25(OH)D measurement techniques were slightly different between the two sites, the investigators decided to analyse data from the two

cohorts separately, instead of pooling the data, so that findings would be internally valid within each cohort.

Statistical methods

Patients hospitalised with a clinical diagnosis of COVID-19 identified by clinical coding (emergency use ICD code U07.1, COVID-19 confirmed by laboratory testing, and code U07.2, COVID-19 diagnosis where laboratory confirmation is inconclusive or not available[15]) were defined as cases. Community cases of COVID-19 were not included, as the outcome measure of interest was hospitalisation with COVID-19. Controls were defined as all other patients who had serum 25(OH)D measurements carried out in the above time period who were not submitted to SARS-CoV-2 testing; none of these patients were hospitalised with COVID-19.

All statistical analyses were carried out using Stata v14.0 (StataCorp LP, College Station, TX, USA). Variables were tested for skewness and kurtosis using the in-built "sktest" function in Stata in order to determine whether they were parametrically or non-parametrically distributed. A non-parametric equality of medians test was used to compare median serum 25(OH)D values between cases and controls. Odds ratios and 95% confidence intervals within the case-control study were estimated. The probability of association was calculated using Pearson's chi-squared test. Logistic regression was used to obtain age- and sex-adjusted odds ratios. Furthermore, analyses were also adjusted for the seasonality of serum 25(OH)D levels, adjusting for samples carried out in the UK - spring/summer months (March through August) versus those carried out in autumn/winter months (September through February). Finally, logistic regression was used to determine any association between

vitamin D status and in-patient mortality from COVID-19, adjusting for age, sex and whether serum 25(OH)D was measured during spring/summer.

RESULTS

Cohort characteristics

Baseline participant characteristics of both primary and validation cohorts are shown in Table 1, and that stratified by cases and controls in Table 2. A total of 58,368 participants were recruited from Lancashire Teaching Hospitals NHS Foundation Trust to the primary cohort, of whom 38,472 (65.9%) were female. Age was non-parametrically distributed in both cohorts. The median age of the primary cohort was 53.2 years [interquartile range, IQR 36.6-69.1 years]. A total of 1,036 (1.8%) participants were hospitalised with COVID-19 and defined as cases. Of the hospitalised patients, 375/1,036 (36.2%) died from COVID-19. Serum 25(OH)D values were non-parametrically distributed in both cohorts. The overall primary cohort median serum 25(OH)D was 50.0 nmol/L [IQR 34.0-66.6 nmol/L]. However, when stratified by case status, median serum 25(OH)D was 50.0 nmol/L [IQR 34.2-66.9 nmol/L] in non-hospitalised patients, versus 35.0 nmol/L [IQR 21.0-57.0 nmol/L] in hospitalised patients, and this difference was significant ($p<0.005$). A total of 607 (58.6%) cases had serum 25(OH)D measured as an in-patient. In hospitalised patients, if serum 25(OH)D was not measured during the in-patient admission, the median time between the test being carried out and admission to hospital was 148 days [IQR 22-265].

A total of 21,234 participants were recruited from Tameside and Glossop Integrated Care NHS Foundation Trust to the validation cohort, of whom 14,527 (68.4%) were female. The median age of the cohort was 55.1 years [IQR 39.8-70.4 years]. A total of 772 (3.6%) participants were hospitalised with COVID-19 and defined as cases. Of the hospitalised

patients, 295/772 (38.2%) died from COVID-19. The overall cohort median serum 25(OH)D was 46.7 nmol/L [IQR 31.3-64.4 nmol/L]. However, when stratified by case status, mean serum 25(OH)D was 47.1 nmol/L [IQR 31.8-64.7 nmol/L] in non-hospitalised patients, versus 33.0 nmol/L [IQR 19.4-54.1 nmol/L] in hospitalised patients, and this difference was significant (p<0.005). A total of 579 (75.0%) cases had serum 25(OH)D measured as an in-patient. In hospitalised patients, if serum 25(OH)D was not measured during the in-patient admission, the median time between the test being carried out and admission to hospital was 51 days [IQR 12-187].

Case-control study

In the primary cohort (Lancashire Teaching Hospitals NHS Foundation Trust), low vitamin D (serum 25[OH]D <50 nmol/L) was associated with increased odds of hospitalisation with COVID-19: OR 2.22 (95% CI 1.93-2.53, p <0.005). This remained significant following adjustment for age, sex and whether serum 25(OH)D was measured in spring/summer (OR_{adj} 2.40, 95% CI 2.10-2.74, p<0.005). This association strengthened when only vitamin D deficient patients (serum 25[OH]D <25 nmol/L) were considered: OR 3.77 (95% CI 3.30-4.30, p<0.005). Again, this remained significant following adjustment for age, sex and spring/summer serum 25(OH)D measurement (OR_{adj} 3.57, 95% CI 3.12-4.08, p<0.005).

These findings were replicated in the validation cohort (Tameside and Glossop Integrated Care NHS Foundation Trust). Again, low vitamin D was associated with increased odds of hospitalisation with COVID-19: OR 2.16 (95% CI 1.83-2.54, p<0.005). This remained significant following adjustment for age, sex and whether serum 25(OH)D was measured in spring/summer (OR_{adj} 2.33, 95% CI 1.98-2.74), p<0.005). Furthermore, this association once

again strengthened when only vitamin D deficient patients were considered: OR 3.36 (95% CI 2.89-3.92, p<0.005). Again, this remained significant following adjustment for age, sex and spring/summer serum 25(OH)D measurement (OR_{adj} 2.98, 95% CI 2.55-3.49, p<0.005). Summary of associations between vitamin D status and hospitalisation risk among both cohorts are shown in Table 3, and the detailed sub-analysis stratified by age, sex and season is available in the table 4.

There was no association between low vitamin D levels and in-patient hospital mortality among patients admitted with COVID-19 in either cohort, both unadjusted and following adjustment for age, sex and spring/summer 25(OH)D measurement.

Discussion

This is one of the largest studies to date to investigate the role of vitamin D in the severity of COVID-19 infection. In this retrospective large-scale case-control observational study, we demonstrated an association between sub-optimal serum 25(OH)D levels and risk of hospitalisation from COVID-19. Our study utilises a large number of community-based patients as well as hospital in-patients and outpatients, and findings replicate across two independent cohorts. We found no association between 25(OH)D levels or vitamin D status and in-patient mortality from COVID-19.

Previous systematic reviews have clearly showed an inverse non-linear association between 25(OH)D concentration and acute respiratory tract infections, including community-acquired pneumonias,[19,20] but these studies were not specifically focused on SARS-CoV-2 infection. Similar to our findings, a study from the UK by Panagiotou *et al* found that low

serum 25(OH)D levels in COVID-19 in-patients were associated with a more severe disease course,[21] but this study included only 134 patients.

Conversely, a study using the UK Biobank looked at 348,598 participants, of whom, only 449 had a confirmed diagnosis of COVID-19 as defined by a positive laboratory test for SARS-CoV-2 (only 0·13% of study population), and did not show any association between 25(OH)D and risk of COVID-19 infection.[22] Additional weaknesses in this study include: heterogeneity in severity and management of COVID-19 cases (likely a mixture of in-patient and community, instead of focusing on COVID-19 cases in only one setting), serum 25(OH)D measurement between 2006 and 2010, and not contemporaneously with COVID-19 infection 10-14 years after recruitment to the UK Biobank, and no mention of validation of 25(OH)D measurement.

In terms of 25(OH)D and COVID-19 disease severity, a study from India of 154 patients admitted to hospital with COVID-19 reported that the mean 25(OH)D level was <30ng/ml (insufficient range), with patients admitted to the intensive care unit and those that died from COVID-19 being more vitamin D deficient than survivors.[23] Another study from Belgium (n=186) reported similar findings of greater deficiency rates in patients with more severe disease.[24] Similarly, a study from Switzerland demonstrated that 25(OH)D concentrations were significantly lower in patients with COVID-19 than in those without the disease.[25]

Other studies have also demonstrated a correlation between vitamin D deficiency and COVID-19 infection, contrary to the study using patients from the UK Biobank. A study from Israel with 7,807 subjects demonstrated that 25(OH)D concentrations were significantly

lower among those who tested positive for COVID-19 than those who were COVID-19 negative.[26] A study from Wuhan, China, showed in a multivariable logistic regression that vitamin D deficiency (<30nmol/L) was significantly associated with COVID-19 severity.[27]

Our study's strengths lie in the usage of relatively recent serum 25(OH)D testing (i.e., carried out within 12 months of in-patient admission with COVID-19), and the use of two large cohorts of patients (n=80,670 combined). We have clearly demonstrated that vitamin D insufficiency and deficiency exponentially increase the risk of the disease by a factor of 2.3-3.6, even after adjustments for age and sex. However, we did not find any association between low 25(OH)D levels or vitamin D status and excess mortality risk, as observed in previous studies.[6,8,11,12]

Vitamin D deficiency has been recognised as a risk factor for developing COVID-19, a disease that has affected over 1 billion people worldwide and has led to over 2.82 million deaths. Vitamin D deficiency is a global problem, more so in countries with colder climates, especially those above the 35th parallel. This is in support of our UK-based study, which also showed that vitamin D deficiency led to more severe COVID-19 that required admission to hospital, increasing the risk approximately three-fold when compared to people with normal 25(OH)D levels.

Vitamin D is a pluripotent secosteroid hormone that is important for bone health, but it is also known to regulate cellular functions throughout the body. Vitamin D, specifically, is of two types: vitamin D2 (ergocalciferol), derived mainly from plant sources, and vitamin D3 (cholecalciferol), which is present in higher animals and constitutes 80-90% of the body's

vitamin D.[28] The role of vitamin D in the immune system could partially explain the relationship between vitamin D deficiency and COVID-19 incidence and disease severity. Vitamin D is anti-inflammatory, and it has been shown to modulate the immune system by upregulating a complex set of proteins and inducing the expression of defence peptides such as cathelicidin and β-defensins.[29]

One of the factors associated with the apparently high mortality risk from the COVID-19 pandemic in the temperate and cold regions of the world compared to the tropics may be vitamin D deficiency. Several previous studies clearly demonstrated high prevalence of low 25(OH)D levels in European populations, especially during winter months.[30-33] As the association between vitamin D deficiency and usual respiratory illnesses/mortality was not sizeable, public health measures for nutritional supplementation, at least during winter months, were not a major agenda, at the time of writing. With the emergence of a mega-pandemic such as COVID-19, with high morbidity and case fatality risk with few treatment options currently, urgent attention to this important issue now becomes crucial.

Vitamin D supplementation has been shown to reduce the risk of respiratory infections in both a previous and recent meta-analyses.[34] We have recently demonstrated that high-dose cholecalciferol treatment was associated with reduced mortality among hospitalised patients with COVID-19 infections.[2] In another study from Spain, Entrenas Castillo *et al* found that in a cohort of patients treated with calcifediol (hydroxylated cholecalciferol or 25-hydroxyvitamin D_3), fewer patients required admission to the intensive care unit.[35]

There has been a lot of discussion on the role of vitamin D in COVID-19. With its effect on macrophage function and innate immunity, vitamin D may alter the disease manifestations of COVID-19. In the absence of highly effective prevention and treatment strategies for the pandemic currently, any medical intervention, including vitamin D supplementation/treatment, becomes relevant. With the easy availability and very economic pricing of the drug, vitamin D supplementation should be an important consideration for deficient populations at risk.

To understand and improve outcomes, risk scores are being devised. Two such scores (QCOVID[36] and OURMAPCN[37]) for risk of hospital admission and mortality from COVID-19 have been developed, and these include patient demographics and biochemical parameters, as well as a range of co-morbidities. However, vitamin D was not included in the analysis, and given widespread, independent findings regarding 25(OH)D levels and risk for severity and mortality from COVID-19, future algorithms should consider inclusion of serum 25(OH)D levels.

We acknowledge certain limitations of our study, which are inherent to the retrospective design of the data collection, being recruitment from only two large district hospitals in the UK, and lack of availability of other confounding factors such as comorbid illnesses that might have increased the hospitalisation rates in patients with COVID-19. However, the large sample size compared to most other published data, examining the association between 25(OH)D levels and COVID-19, as well as validation between two independent cohorts, make our data unique. As we obtained data of COVID-19 cases from respective hospital admissions registers from both sites, we were unable to assess the risk of

asymptomatic COVID-19 in people with vitamin D deficiency in the community. We were also unable exclude other potential confounding factors (such as obesity) which are associated with vitamin D deficiency and higher morbidity from COVID-19.[38,39] Even with these limitations, our observations may have important public health implications for planning and policy-making to prevent and treat COVID-19, the enigmatic disease that still threatens normal human life across the globe.

Conclusions:

Vitamin D insufficiency or deficiency is associated with 2.3-3.6 times higher risk of severe SARS-CoV-2 infection necessitating hospital admission. However, there is no association between vitamin D deficiency and excess mortality in COVID-19. Urgent action is required to address the high prevalence of vitamin D deficiency that increases COVID-19-related morbidity. Future studies should also investigate any potential role of vitamin D sufficiency in the prevention of SARS-CoV-2 infection.

Data availability: Full data of this research work is available with the corresponding author, and can be viewed by interested parties on request.

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Tables

Table 1. Participant characteristics of both primary and validation cohorts.

Participant characteristics	**Lancashire Teaching Hospitals NHS Foundation Trust** (n = 58,368)	**Tameside and Glossop Integrated Care NHS Foundation Trust** (n = 21,234)
Age (years), median [interquartile range, IQR]	53.2 [36.6-69.1]	55.1 [39.8-70.4]
Female sex, n (%)	38,472 (65.9)	14,527 (68.4)
Hospitalised patients, n (%)	1036 (1.8)	772 (3.6)
In-patient deaths, n (%)	375 (36.9)	295 (38.2)
Serum 25(OH)D (nmol/L), median [IQR]	50.0 [34.0-66.6]	46.7 [31.3-64.4]
Time (days) between 25(OH)D measurement and admission to hospital (hospitalised participants only), median [IQR]	148 [22-265]	51 [12-187]

Table 2. Participant characteristics of both primary and validation cohorts, stratified by cases and controls.

Participant characteristics	**Cases**	**Controls**
Primary cohort: Lancashire Teaching Hospitals NHS Foundation Trust (n = 59,368)		
Age (years), median [IQR]	73.7 [60.3-82.6]	52.8 [36.3-68.7]
Female sex, n (%)	472 (45.6)	38,000 (66.3)
Serum 25(OH)D (nmol/L), mean (SD)	35.0 [21.0-57.0]	50.0 [34.2-66.9]
Validation cohort: Tameside and Glossop Integrated Care NHS Foundation Trust (n = 21,234)		
Age (years), median [IQR]	72.5 [60.1-81.5]	54.5 [39.3-69.7]
Female sex, n (%)	345 (44.7)	14.182 (69.3)
Serum 25(OH)D (nmol/L), mean (SD)	33.0 [19.4-54.1]	47.1 [31.8-64.7]

Table 3. Summary of associations between vitamin D status and hospitalisation, both cohorts.

Variable of interest	*Primary cohort*		*Validation cohort*	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Serum 25(OH)D <50 nmol/L	2.22 (1.93-2.53)	<0.005	2.16 (1.83-2.54)	<0.005
Serum 25(OH)D <50 nmol/L, adjusted	2.40 (2.10-2.74)	<0.005	2.33 (1.98-2.74)	<0.005
Serum 25(OH)D <25 nmol/L	3.77 (3.30-4.30)	<0.005	3.36 (2.89-3.92)	<0.005
Serum 25(OH)D <25 nmol/L, adjusted	3.57 (3.12-4.08)	<0.005	2.98 (2.55-3.49)	<0.005

Where odds ratios have been adjusted, these have been adjusted for age, sex and whether serum 25(OH)D measurement was carried out in UK spring/summer months (March through August).

Table 4

A. Primary cohort sub-analysis

Serum 25(OH)D <50 nmol/L and association with hospitalisation

Sub-group	OR (95% CI)	p-value
Female	2.15 (1.78-2.61)	<0.005
Male	2.10 (1.75-2.52)	<0.005
Age ≥60 years	2.46 (2.12-2.86)	<0.005
Age <60 years	2.68 (2.02-3.55)	<0.005
25(OH)D measured in spring/summer	2.14 (1.69-2.72)	<0.005
25(OH)D measured in autumn/winter	2.19 (1.87-2.57)	<0.005

Serum 25(OH)D <25 nmol/L and association with hospitalisation

Sub-group	OR (95% CI)	p-value
Female	3.56 (2.91-4.35)	<0.005
Male	3.59 (3.00-4.28)	<0.005
Age ≥60 years	3.68 (3.15-4.31)	<0.005
Age <60 years	4.11 (3.17-5.32)	<0.005
25(OH)D measured in spring/summer	4.54 (3.58-5.75)	<0.005
25(OH)D measured in autumn/winter	3.42 (2.91-4.01)	<0.005

B. Validation cohort sub-analysis

Serum 25(OH)D <50 nmol/L and association with hospitalisation

Sub-group	OR (95% CI)	p-value
Female	2.15 (1.70-2.72)	<0.005
Male	1.92 (1.54-2.40)	<0.005
Age ≥60 years	2.47 (2.06-2.97)	<0.005
Age <60 years	2.39 (1.71-3.36)	<0.005
25(OH)D measured in spring/summer	2.30 (1.71-3.10)	<0.005
25(OH)D measured in autumn/winter	2.05 (1.69-2.48)	<0.005

Serum 25(OH)D <25 nmol/L and association with hospitalisation

Sub-group	OR (95% CI)	p-value
Female	3.30 (2.62-4.16)	<0.005
Male	2.93 (2.38-3.59)	<0.005
Age ≥60 years	3.19 (2.67-3.82)	<0.005
Age <60 years	3.43 (2.53-4.64)	<0.005
25(OH)D measured in spring/summer	4.28 (3.25-5.64)	<0.005
25(OH)D measured in autumn/winter	3.00 (2.50-3.61)	<0.005