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Original Article

Children with cystic fibrosis demonstrate no respiratory immunological, infective or physiological, consequences of vitamin D deficiency

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Abstract

Background: Vitamin D has health benefits in many respiratory diseases but the evidence in CF is unclear. Induction of the antimicrobial peptides cathelicidin (LL37) and human-beta-defensin-2 (HBD-2) may be the mechanism of any benefit. We hypothesised that antimicrobial peptide levels would be decreased, and airway infection and inflammation greater, in CF children with vitamin D deficiency. The objective of the study was to explore relationships between vitamin D, LL37 and HBD-2, and airway infection, inflammation and physiology in children with CF.
Methods: Bronchoalveolar lavage (BALF) and blood were obtained from children undergoing fibreoptic bronchoscopy. Serum vitamin D, BALF HBD-2 and LL37, cultured bacteria and inflammatory markers were measured. Clinical parameters were recorded.
Results: 113 patients with CF, 23 with non-CF chronic suppurative lung disease (CSLD) and 6 healthy controls were included. We found no relationship between serum vitamin D and BALF HBD-2 or LL-37. There were no differences in infective or inflammatory markers between vitamin D sufficient and deficient groups. Vitamin D deficient patients (<50 nmol/L) did not have a worse FEV_1 (CF: 66 (58–71)% vs. 71.5 (61–76)%, ns; non-CF CSLD: 69 (36–88)% vs. 70 (62–95)%, ns).
Conclusions: In the first bronchoscopic study exploring this question, we demonstrate that vitamin D deficiency is not associated with immunological, infective or clinical markers of disease severity in patients with CF or CSLD.

Keywords: Cystic fibrosis; Vitamin D; Innate immunity; Children

1. Introduction

Recent evidence has pointed to vitamin D having a role in respiratory health, in addition to its classical role in bone health [1]. Vitamin D is obtained either from the diet or by the conversion of the steroid 7-dehydrocholesterol, present in the skin, following exposure to UV-light. Vitamin D receptors have been identified on many immune cells [2]. Activated vitamin D binds to vitamin D response elements (VDRE), which have been identified on genes encoding innate antimicrobial peptides, LL-37 and human-β-defensin-2 (HBD-2) [3], which are effective against many pathogens [4]. In-vitro expression of these peptides is up-regulated by vitamin D [5,6]. Vitamin D also has numerous effects on the adaptive immune system, although the exact pathways are not fully understood [2].

Fat malabsorption in cystic fibrosis (CF) may predispose to vitamin D deficiency. People with CF suffer from frequent lower respiratory tract infections which contribute to ongoing inflammation, leading to decline in respiratory health, reduced pulmonary function and eventual death from respiratory failure. Whilst adequate levels of vitamin D have been shown to be

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important in tuberculosis, influenza, other lower respiratory tract infections [7,8] and asthma [9], the evidence for a role in CF lung disease is limited. The largest study to date found that vitamin D had a positive, but very weak, correlation with forced expiratory volume in 1 s (FEV_1) and a negative correlation with serum IgG [10] but results of multiple smaller studies are conflicting [11–16]. A systematic review [17] concluded that there was a weak positive correlation between serum vitamin D and respiratory health in CF. A study looking at 130 children with CF found more exacerbations in vitamin D deficient patients (<50 nmol/L) than insufficient (50–75 nmol/L) or sufficient (≥75 nmol/L) patients in the 15–18 year old age groups, but no difference in the younger age groups [14] and another study of 148 children with CF found lower vitamin D in those infected with *Pseudomonas aeruginosa* [18].

Here we evaluated the respiratory health of CF and non-CF chronic suppurative lung disease (CSLD) patients and the relationship to serum vitamin D. We hypothesised that worse lung health in subjects with vitamin D deficiency would be explained by lower levels of vitamin D-responsive innate polypeptides and worse airway infection and inflammation.

2. Methods

2.1. Patients

We studied children ≤16 years of age undergoing clinically-indicated fibreoptic bronchoscopy (FOB). Subjects had CF, non-CF CSLD: primary ciliary dyskinesia (PCD, diagnosed on conventional criteria) or bronchiectasis (diagnosed radiologically). FOB was performed at CF diagnosis and for decline in respiratory status in CF and non-CF CSLD. Other children undergoing FOB for reasons such as upper airway examination were included as healthy controls (HC) only if they had macroscopically normal airways, no bacteria, fungi or viruses identified and a bronchoalveolar lavage fluid (BALF) neutrophil differential of ≤4%.

2.2. Sample collection and processing (see OLS)

FOB was performed as previously described [19]; Blood and BALF for cell count were stored on ice and processed within 4 h.

BALF cytokines were measured by sandwich immunoassay (Meso Scale Discovery, Rockville) and HBD-2 and LL-37 by ELISA (Phoenix Pharmaceuticals, USA and Hycult Biotech, Netherlands respectively). Serum 25(OH)D was measured using mass spectrometry coupled with high-performance liquid chromatography. For convenience we refer to 25(OH)D as "vitamin D".

Microbiological culture results, from BALF, and previous sputum and cough swab samples, were obtained from the hospital microbiology laboratory. PCR was performed for respiratory syncytial virus, adenovirus, parainfluenza 1, 2 and 3, influenza A and B, human metapneumovirus and rhinovirus.

Clinical data were obtained from electronic hospital records and from the national CF registry, "Port CF" from the time of the FOB, the annual assessment closest to this time point, and the annual assessments before and after, allowing 3 years' data to be reviewed (see OLS). Spirometry, performed according to ATS/ERS standards and expressed as percent predicted, was collected from the same sources.

2.3. Definition of vitamin D deficiency

Vitamin D concentrations were as continuous and categorical data. For the latter, data are presented based on the common clinical cut off of 50 nmol/L [20,21]; however this level is based on maintaining bone health not immunological health [22,23]. As different levels for vitamin D deficiency have been suggested, to ensure that signals were not being missed, post-hoc analyses were undertaken using alternative cut-off values (OLS Table 1).

2.4. Statistical analyses

The sample size was opportunistic, as a) only clinically-indicated bronchoscopies were included for ethical reasons and b) there were no data in this population to inform a power calculation. Non-parametric analysis was used (see OLS) and data are presented as median and 95% confidence intervals. Fishers' exact test was used for group comparisons. Multiple linear regression was performed using SPSS v21 (IBM Inc., USA) statistical package to determine significant relationships, if any, to the BALF and serum components measured. The factors tested in all cases were age, gender, total vitamin D level, genotype (phe508del/phe508del versus the rest) and winter (October to March inclusive) versus summer (April to September inclusive). Forward conditional logistic regression with *Staphylococcus aureus* or *Pseudomonas aeruginosa* bacterial isolation or not as the binary outcome was performed using age and total vitamin D level as factors.

As innate immunity is thought to be most important in young children, post-hoc analyses were also performed excluding all children over 2 years of age, and including only the children 2 years of age and above.

In view of the multiple comparisons undertaken, we chose a priori to reject the null hypothesis at $p < 0.01$.

3. Results

142 patients (113 CF, 23 non-CF CSLD and 6 healthy controls) were included in the study (Table 1).

3.1. Vitamin D levels

Despite 91% of CF subjects being prescribed fat-soluble vitamin supplements, 41/113 (36%) were vitamin D deficient (<50 nmol/L). Median (95%CI) values were similar to those of the healthy controls; 57 (52:66) and 57 (24:74) nmol/L respectively, (Table 2 and OLS Fig. 1). In contrast, the patients with non-CF bronchiectasis, in whom vitamin D is not routinely supplemented, had lower serum levels (42 (26:51) nmol/L $p < 0.01$ vs. CF); 70% of them were deficient

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Table 1
Patient demographics of the 3 groups included in the study. There were no statistically significant differences between the groups except that the proportion of children under the age of 1 year was higher in the CF group than the 2 other groups ($p < 0.01$). Despite clear numerical differences in FEV_1 between the CF group and the healthy controls this did not reach statistical significance, presumably due to the small numbers in the control group.

	CF	Non-CF CSLD	Healthy	p value
n	113	23	6	
Age years	7.8	7.8	12.4	$p < 0.01$
Median (range)	(0.1–17.6)	(2.8–15.5)	(10.5–15.4)	
Sex	47 (42)	12 (52)	2 (33)	ns
n (%) male				
F508del homozygous	51 (46%) [a]	Not done	Not done	
Pancreatic insufficiency	99 (88%)	n/a	n/a	
Vitamin supplements	102 (91%) [b]	Not available	Not available	
FEV_1 % predicted	66	69	106	ns
Median (range)	(30−101)	(25–95)	(80–131)	
	n = 59	n = 17	n = 2	
BMI z-score Median (range)	−0.5	0.6	1.1	$p < 0.01$
	(−0.4–2.6)	(−2.5–2.7)	(0.3–2.7)	
	n = 98	n = 22	n = 4	
% with *Staphylococcal aureus* on BAL	15	4	0	
% with *Pseudomonas aeruginosa* on BAL	12	12	0	
% culture positive on BAL	56	43	0	

n/a not available.
[a] 3 pts missing data.
[b] 1 pt data missing.

(<50 nmol/L; $p < 0.01$ vs. CF). Within the CF group, pancreatic insufficient patients did not have significantly lower levels than their pancreatic sufficient counterparts (58 (53:66) nmol/L) vs. (51 (25:79), $p > 0.01$). There was an inverse relationship between vitamin D and age ($r = -0.35$, $p < 0.001$) (see OLS).

3.2. Antimicrobial peptides

BALF levels of LL-37 and HBD-2 were similar in the 3 patient groups (Table 2). Contrary to our hypothesis, no relationship was seen between BALF levels of either

Table 2
Vitamin D concentration, cellular inflammatory markers and antimicrobial peptide levels for the 3 patient groups. Results shown are median (95% CI of the median) and p-values refer to the Dunn's multiple comparison test. Patients with non-CF CSLD had lower levels of vitamin D than the CF patients and the healthy controls ($p < 0.01$). The CF group had higher BALF absolute cell count ($p < 0.01$), neutrophil differentials and neutrophil count than the healthy controls ($p < 0.001$). There was no difference in blood neutrophils between the 3 different groups. IL-12p70 was higher in CF than healthy controls; IL-6, IL-8 and TNF-α were also higher in CF than healthy controls but these did not meet our predefined cut off of 0.01 on multiple comparison testing.

	CF	Non-CF CSLD	Healthy	p value
Serum 25(OH)D (nmol/L)	57 (52–66)	42 (26–51)	57 (24–74)	$p < 0.01$ [*]
Blood neutrophils ($\times 10^9$/L)	4.3 (3.7–4.9)	4.6 (3.6–5.7)	3.9 (2.6–4.6)	ns
BALF absolute count ($\times 10^3$)	620 (500–850)	364 (115–540)	143 (70–270)	$p < 0.01$ [†]
BALF neutrophil differential (%)	31 (19.0–41.7)	14.5 (2.0–46.3)	1.4 (0.3–2.7)	$p < 0.001$ [†]
BALF neutrophil count ($\times 10^3$)	166 (45.6–306.9)	16.5 (4.2–346.5)	2.8 (0.2–5.1)	$p < 0.001$ [†]
BALF LL-37 (ng/mL)	0.5 (0.3–0.75)	0.4 (0.3–0.8)	0.3 (0.3–0.6)	ns
BALF HBD-2 (pg/mL)	149 (115.8–202.6)	51 (<15.6–170.5)	120 (<15.6–250)	ns
BALF IL-2 (pg/mL)	0.41 (0.10–0.59)	0.27 (0.01–0.53)	0.07 (0.00–0.44)	ns
BALF IL-6 (pg/mL)	13.25 (7.32–22.16)	2.73 (0.92–35.07)	2.43 (0.511–5.76)	$p = 0.03$ [†]
BALF IL-8 (pg/mL)	1363 (728.3–2382)	857.5 (66.17–4122)	113.7 (30.6–378.9)	$p = 0.03$ [†]
BALF IL-10 (pg/mL)	0.50 (0.31–1.17)	0.37 (0.13–2.39)	0.51 (0.00–0.95)	ns
BALF TNF-α (pg/mL)	1.53 (0.73–3.29)	0.82 (0.04–13.3)	0.13 (0.08–0.45)	$p = 0.04$ [†]
BALF IFN-γ (pg/mL)	0.07 (0.00–0.33)	0.06 (0.00–1.22)	0.005 (0.00–0.52)	ns
BALF GM-CSF (pg/mL)	0.50 (0.18–0.83)	0.44 (0.12–0.71)	0.44 (0.04–2.48)	ns
BALF IL-12p70 (pg/mL)	0.19 (0.10–0.42)	0.17 (0.03–1.16)	0.00 (0.00–0.08)	$p = 0.007$ [†] $p = 0.03$ [$]
BALF IL-1β (pg/mL)	14.87 (8.47–32.54)	4.50 (1.23–181.1)	7.61 (1.32–14.68)	ns

[*] CSLD lower than both CF and healthy controls.
[†] CF greater than healthy controls.
[$] CSLD greater than healthy controls.

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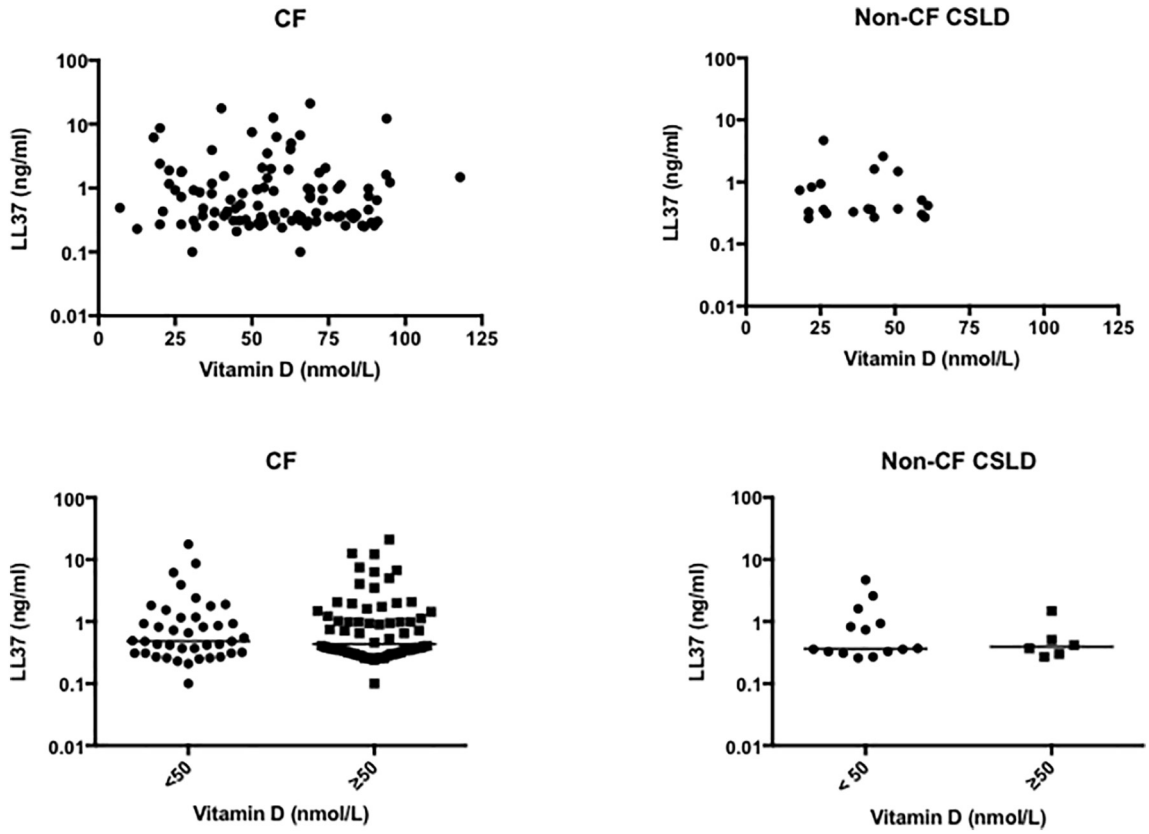

Fig. 1. Vitamin D and LL-37. No correlation was seen between serum 25(OH)D and BALF LL-37 in either CF (r = −0.06, ns) or non-CF CSLD (r = −0.02, ns) patients (a, b). In addition, no difference was observed in either group between vitamin D deficient and vitamin D sufficient patients (median (CI): CF patients; 0.49 (0.37–0.86) ng/ml vs. 0.44 (0.37–0.94) ng/mL, ns. Non-CF CSLD patients; 0.37 (0.31–1.62) ng/mL vs. 0.40 (0.27–1.49 ng/mL, ns) (c, d).

antimicrobial peptides and serum vitamin D (Figs. 1 and 2). LL-37 was higher in patients with bacterially infected BALF (OLS Fig. 2) and in CF patients LL-37 correlated positively with other markers of inflammation, including cell count ($r = 0.7$; $p < 0.0001$), neutrophil differential ($r = 0.5$; $p < 0.0001$) and several of the BALF pro-inflammatory cytokines (OLS Table 2 and OLS Figs. 3 and 4). For non-CF patients, a similar but non-statistically significant trend was seen. These correlations were not seen for HBD-2 in any group (OLS Fig. 5). No correlation was seen between either LL-37 or HBD-2 and FEV_1 or FVC (OLS Fig. 6).

3.3. Cellular and soluble markers of inflammation

No relationships between any cellular or soluble BALF inflammatory markers and vitamin D were seen (Fig. 3 and Table 3). As expected, BALF total inflammatory cell counts and neutrophil differential counts were significantly increased ($p < 0.01$) in CF patients compared with healthy controls (Table 2). CF patients also had a higher BALF cell count than the CSLD group ($p = 0.027$) and the CSLD a higher neutrophil differential than the healthy control group ($p = 0.033$) although these did not reach our statistical cut-off, of $p < 0.01$. Equally, BALF IL-6 and IL-8 were higher in CF patients than healthy controls, but again, these did not reach our pre-defined statistical cut-off on 3-way analysis (Table 2). The cellular and soluble markers of inflammation correlated positively with infection status (OLS Fig. 7), as expected, acting as a useful positive control.

3.4. Infection

Overall, there was no relationship between vitamin D and BALF bacterial culture status (OLS Figs. 1 and 8). A more detailed analysis of vitamin D by infection status was undertaken only for CF patients, because of the low numbers in the other groups; we focussed on *P. aeruginosa* and *S. aureus* as the predominant bacteria in this group as well as viral PCR. There was no relationship between vitamin D levels and isolation of *Pseudomonas aeruginosa* in any of the 3 ways of exploring this; isolation from BALF, from surveillance swabs over a one year follow up period, or the 3 year follow up period. For *Staphylococcus aureus*, there was an initial apparent relationship in the 1-year follow-up period but not in the other 2 ways of looking at this; this apparent relationship disappeared when age was assessed as an independent variable. We also looked at correlations between vitamin D levels and any positive bacterial or fungal culture; again, no relationship was seen in any of the 3 ways of exploring the data. BALF viral PCR analysis was available for 24/113 (21%) of the CF group; there was no association with either vitamin D deficiency or LL-37/HBD2 levels (OLS Table 3).

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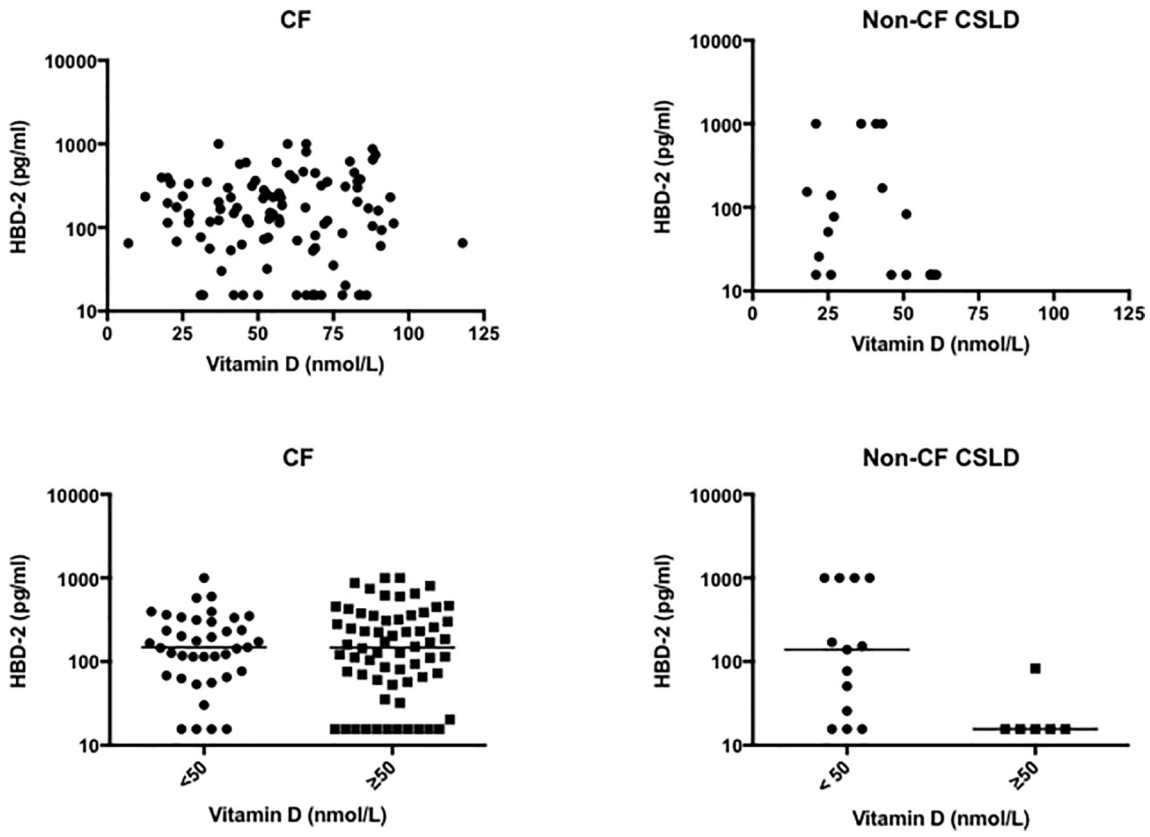

Fig. 2. Vitamin D and HBD-2. No correlation was seen between serum vitamin D and BALF HBD-2 in either CF (r_s=−0.02, ns) or non-CF CSLD (r_s=−0.4, ns) patients (a, b). In addition, no difference was observed in either group between vitamin D deficient and vitamin D sufficient patients (median (95% CI): CF patients; 149 (115–235) pg/ml, ns. Non-CF CSLD patients; 139 (15.6–1002) pg/ml vs. 15.6 (15.6–83) pg/ml ns (c, d). Values above and below detection ranges were excluded with no alteration to outcomes. Values were therefore included in analyses and have been plotted at the lower and upper limits of detection.

3.5. Clinical parameters

Spirometry was available for 60/113 (53%) CF patients and 16/23 (70%) non-CF CSLD group but not for the others either because they were too young (CF = 50, CSLD = 2) or because spirometry was not performed sufficiently close to the time of the bronchoscopy (CF = 3, CSLD = 4). Spirometry was performed in just 2 of the healthy controls and therefore analysis of this patient group was not performed. There was no correlation between vitamin D and spirometry (FEV_1 or forced vital capacity (FVC)) in CF or non-CF CSLD groups, analysed either separately or combined, and neither parameter differed between vitamin D sufficient and deficient groups (Fig. 4 and Table 4). This lack of relationship persisted if the spirometric values from the previous or following year's AA were analysed, or if all 3 were meaned in the CF cohort (OLS Fig. 9). No relationship was seen between vitamin D level and the number of days on intravenous antibiotics (IVAB) or height, weight or BMI z-score (Table 4). This was consistent irrespective of whether the year of the FOB, or the periods before and after were assessed (data not shown).

Post-hoc analyses of children <2 years of age, and of children 2 years did not alter the conclusions (data not shown).

4. Discussion

To our knowledge this is the first time that bronchoscopic inflammatory and innate defence markers have been evaluated against vitamin D levels in the CF population and provide evidence of a lack of inflammatory role of vitamin D in the CF airway. We did not find any association between serum vitamin D and BALF LL-37 and HBD-2, airway infection or inflammation, in children with CF and the lack of physiological and clinical correlations further support these findings. Over half of our CF population were vitamin D deficient, despite most (91%) being prescribed supplements. Unexpectedly, the non-CF CSLD group had a significantly lower vitamin D level than either CF children or healthy controls ($p < 0.01$). 67% of non-CF CSLD were vitamin D insufficient (<50 nmol/L) compared with 34% of CF patients and 33% of healthy controls; although the former is a small group, the effect did not appear to be accounted for by ethnicity or age and merits further study. In this group also, and similarly to CF, vitamin D level was not associated with severity of airway disease assessed by FEV_1. However, the fact that the non-CF CSLD group, who would be expected to have a better prognosis in fact had lower Vitamin D levels than CF may lend support to our conclusion that Vitamin D

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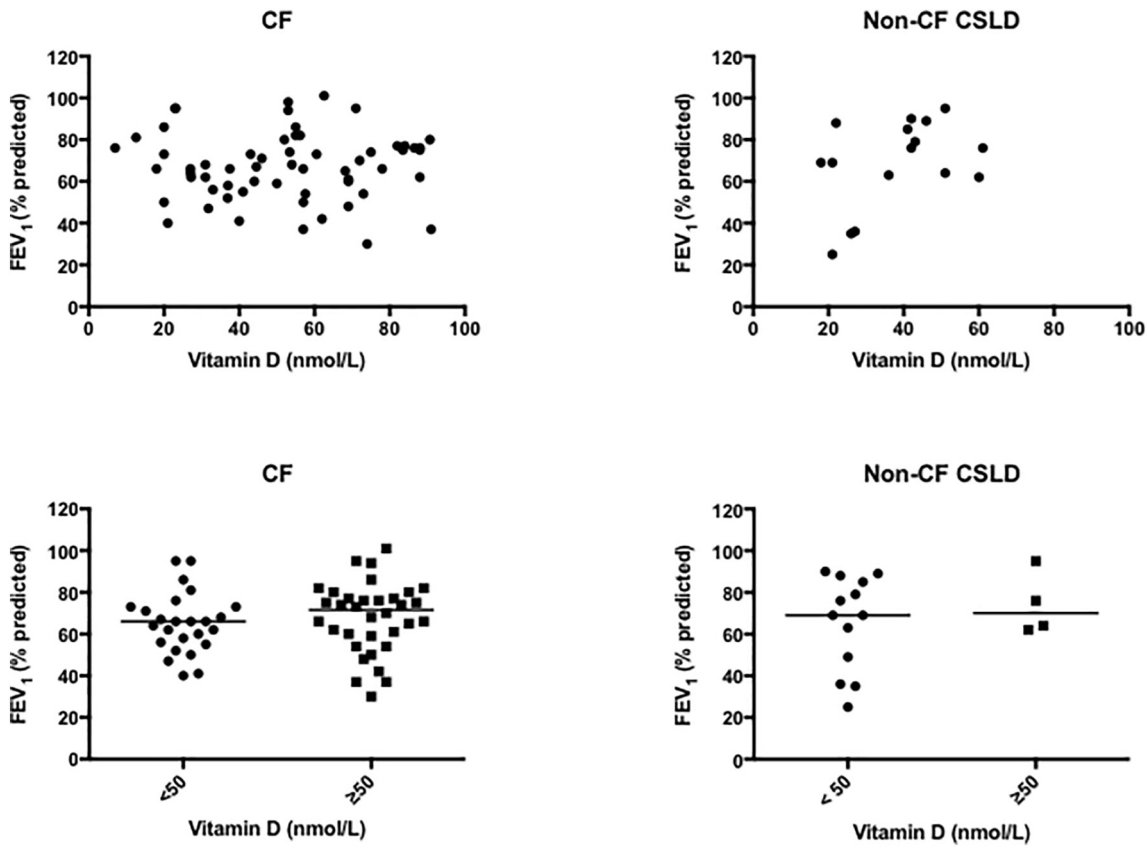

Fig. 3. No correlation was seen between serum vitamin D and BALF cell count in either CF (r = −0.2, ns) or non-CF CSLD (r = −0.007, ns) patients (a, b). In addition, no difference was observed in either group between vitamin D deficient and vitamin D sufficient patients (median (CI): CF patients; 652 (500–1220) vs. 528 (390–850) × 10^3, ns). Non-CF CSLD patients; 364 (200–500) vs. 460 (60–1240) × 10^3, ns (c, d). A similar picture was seen with BALF neutrophil differential and serum neutrophils.

deficiency is not an important determinant of lung health in either group.

We found a weak, but statistically significant, inverse relationship between vitamin D and age in CF. There are a number of reasons why younger children may have higher levels of vitamin D. Several foods, including formula milks more likely to be ingested by younger children, are fortified with vitamin D. Younger children may be more compliant with their medications, as these are administered by parents [28]. Post-hoc analyses confirmed there was no change in our findings when children under the age of 2 years were excluded from the group. Perhaps more importantly, post-hoc analyses of CF patients <2-years-old were performed. Innate immunity is thought to be of particular importance in the early years, before the development of adaptive immunity but even in this age group, vitamin D level did not correlate with markers of inflammation.

One strength of this study is that the lack of mechanistic differences was confirmed by clinical findings. In order to

Table 3
Vitamin D and cellular makers of inflammation, median (95% CI of median). There was no relationship between vitamin D level and any of the cellular markers of inflammation for either CF or non-CF CSLD patients. Although not statistically significant, it was interesting to note that CF patients with lower vitamin D had a trend towards more inflammation whilst the opposite was true for non-CF CSLD patients.

	N	Vitamin D < 50 nmol/L	Vitamin D > 50 nmol/L	p value
CF patients				
BALF total cell count (×10^3)	101	652 (500–1220)	528 (390–850)	ns
BALF neutrophil differential (%)	84	36 (20–56)	25 (15–50)	ns
BALF neutrophil count (×10^3)	76	207 (40–521)	102 (43–346)	ns
Blood neutrophil count (×10^9/L)	102	4.9 (3.8–6.5)	4.2 (3.4–4.9)	ns
Non-CF CSLD				
BALF total cell count (×10^3)	16	364 (200–500)	460 (60–1240)	ns
BALF neutrophil differential (%)	18	13.4(2.0–79)	29.3 (0.0–72)	ns
BALF neutrophil count (×10^3)	13	8.6(3.3–347)	299 (16.4–582)	ns
Blood neutrophil count (×10^9/L)	18	5.1 (3.6–5.7)	2.7 (2.2–11.8)	ns

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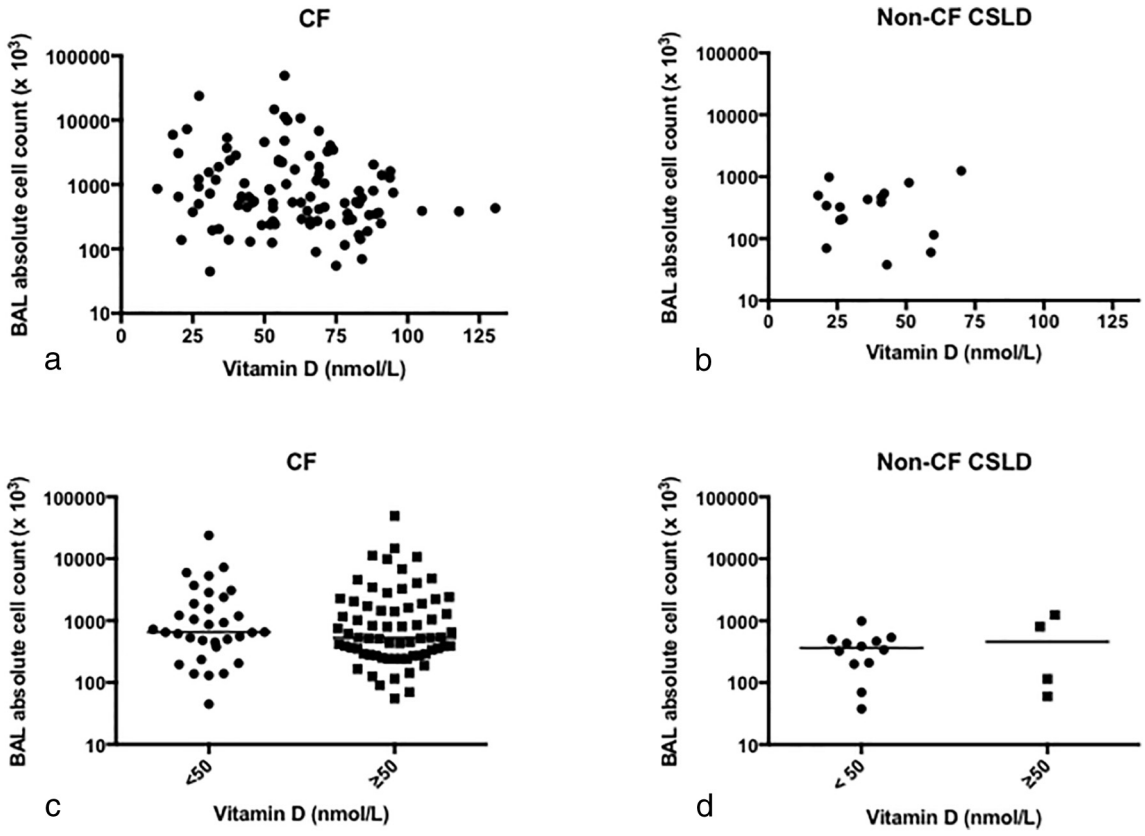

Fig. 4. Figure a and b show the correlation between serum vitamin D and FEV_1 for CF (a) and CSLD (b) patients. Figures c and d show the FEV_1 for vitamin D deficient vs. vitamin D sufficient patients. There was no correlation between vitamin D and FEV_1 (a and b) for either group (CF: r = 0.03, ns; non-CF CSLD: r = 0.3, ns) and when comparing vitamin D sufficiency and deficiency (c and d), no difference was seen (median (CI) vitamin D in CF: 66 (58–71)% vs. 71.5 (61–76)%, ns; non-CF CSLD: 69 (36–88)% vs. 70 (62–95)%, ns).

ensure a signal was not missed, and as the optimal vitamin D level for respiratory health is unknown, different cut-off values were used to define cohorts, with no change in outcome. The discrepancy in values used by some groups to determine vitamin D insufficiency [8,25–27] illustrates the lack of consensus as to an appropriate vitamin D level that should be accepted.

To maximise the reliability of the conclusions, we studied large numbers (113) of CF patients with a wide range of disease severity. However, the numbers in the non-CF CSLD and HC group were smaller due to the relatively less frequent FOBs being performed in these patients. A further limitation is that few CF patients beyond the first year of life undergo FOB at a time of clinical stability; patients are recruited opportunistically, when having clinically-indicated bronchoscopy, biasing towards the exacerbating state. It is possible therefore that these data cannot be directly extrapolated to the stable CF lung. However, as CF is a condition with frequent exacerbations,

Table 4
The table shows Vitamin D levels and clinical parameters for CF and non-CF CSLD patients. Median (95% CI of median) FEV_1, number of days per year spent on intravenous antibiotics (IVAB) and BMI centile are shown. There was no relationship demonstrated between serum vitamin D level and FEV_1, FVC (figures not shown), the number of days on IVAB or BMI centile.

	Vitamin D < 50 nmol/L	Vitamin D > 50 nmol/L	Difference between 2 groups (p value)	Correlation with serum vitamin D
CF patients				
FEV_1 % predicted median	66.0	71.5	ns	r = 0.03
(range)	(58–71)	(61–76)		ns
Days on IVAB	14	12	ns	r = −0.05
Median (range)	(0–56)	(0–18)		ns
BMI z-score	−0.3	−0.6	ns	r = −0.19
Median (range)	(−3.0–2.1)	(−4.0–2.6)		ns
Non-CF CSLD				
FEV_1 % predicted median (range)	69.0	70.0	ns	r = 0.34
	(36–88)	(62–95)		ns

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which directly impact chronic respiratory health, we consider these findings nevertheless to be relevant. Microbiological culture status at the time of bronchoscopy may be affected by the use of antibiotics and sampling errors [29]; future studies could use molecular techniques if the relationship of Vitamin D on the microbiome is to be studied.

We found no relationship between spirometry and vitamin D, unlike the findings of a large study in Scandinavia [11], which found a positive correlation between vitamin D and FEV_1 ($r^2 = 0.308$, $p = 0.025$). However, the scatter was wide and we would question how clinically meaningful these data are on an individual basis. By exploring the spirometry of patients, not just at the time of the vitamin D measurement, but over a 3-year period, our conclusion that there is no relationship is strengthened. In addition, other clinical parameters such as BMI and days on IVAB, showed no relationship to vitamin D. At present we cannot account for the difference between these two studies. However, in our study, we looked at mechanistic difference (antimicrobial peptides) as well as clinical effects and found no difference in either.

A study conducted in infants and pre-school children with CF [30] found that vitamin D deficiency was associated with increased risk of *S. aureus* respiratory infection, whilst a second study found that patients with *P. aeruginosa* had a lower median serum vitamin D than those without [18]. However, there were no downstream inflammatory consequences of this apparent increased bacterial infection; the authors of one of these studies [18] commented themselves that whilst the difference was statistically significant it may have been of doubtful *clinical* significance as median values in the groups were so close. They also commented that even if an association did exist, this could be a reflection of medication adherence. Although we did find more *S. aureus* and *P. aeruginosa* at some time points within the study, in logistical regression models these relationships failed to hold up once age had been accounted for. The lack of this relationship being seen in all settings and its relationship to age make us confident that at least in our cohort, there is no true relationship between vitamin D and bacterial infection. In keeping with both of the previous studies [18,30] we did not find any association between vitamin D and clinical parameters or inflammatory markers.

The presence of a vitamin D response element in the promoters of camp and defB2 (the genes encoding LL-37 and HBD-2) suggests that vitamin D could play a role in LL-37/HBD-2 expression; indeed in-vitro data have shown increased expression of LL-37, and to a lesser extent HBD-2, following vitamin D administration to CF cells [5]. One small RCT involving 30 CF patients treated with either intramuscular vitamin D or placebo at the time of a pulmonary exacerbation found no difference in plasma LL-37 levels between the groups [31]. Similarly to that study, we found no effect of vitamin D on LL-37 level, contrasting with in-vitro data of vitamin D induced expression of LL-37 in CF cells. Another in-vitro study found that CF bronchial epithelial cells had an impaired ability to activate vitamin D [32].

It is possible that the expression of LL-37 and HBD-2 is induced by vitamin D in vivo, but that these peptides are degraded in the proteolytic environment of the CF airway and therefore no relationship is subsequently apparent with peptide levels. However, LL-37 correlated positively with other markers of inflammation including neutrophil numbers lending validity to the levels measured. If vitamin D was exerting an effect on the innate immune system, regardless of the mechanism of action, we would expect to see downstream consequences with decreased cellular markers of inflammation and decreased pro-inflammatory cytokines in patients with higher levels of vitamin D. This was not the case in our study and no relationship was demonstrated between vitamin D and the BALF innate markers. It is possible that vitamin D affects other defence proteins which have not been explored here, although in the absence of detectable down-stream consequences, any such mechanisms may be of questionable clinical relevance. If there is a biological effect of vitamin D on any of these pathways, the benefit is not translated to a clinical one.

In conclusion, our findings demonstrate that there is no relationship between serum levels of vitamin D and BALF levels of either HBD-2 or LL37, and no effect on airway inflammation and no detectable clinical or physiological effects of vitamin D deficiency.

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Competing interests

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2018.02.011.

References

[1] Bergman P, Lindh AU, Bjorkhem-Bergman L, et al. Vitamin D and respiratory tract infections: a systematic review and meta-analysis of randomized controlled trials. PLoS One 2013;8(6):e65835.
[2] Di RM, Malaguarnera M, Nicoletti F, et al. Vitamin D3: a helpful immuno-modulator. Immunology 2011;134(2):123–39.
[3] Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med 2011;3(104):104ra02.
[4] Tecle T, Tripathi S, Hartshorn KL. Review: defensins and cathelicidins in lung immunity. Innate Immun 2010;16(3):151–9.
[5] Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173(5):2909–12.
[6] Yim S, Dhawan P, Ragunath C, et al. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007;6(6):403–10.

[7] Walker VP, Modlin RL. The vitamin D connection to pediatric infections and immune function. Pediatr Res 2009;65(5 Pt 2):106R-13R.
[8] Williams B, Williams AJ, Anderson ST. Vitamin D deficiency and insufficiency in children with tuberculosis. Pediatr Infect Dis J 2008;27 (10):941–2.
[9] Sutherland ER, Goleva E, Jackson LP, et al. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med 2010;181(7):699–704.
[10] Pincikova T, Nilsson K, Moen IE, et al. Inverse relation between vitamin D and serum total immunoglobulin G in the Scandinavian Cystic Fibrosis Nutritional Study. Eur J Clin Nutr 2011;65(1):102–9.
[11] Beattie S, Srivastava T, Geheb J, et al. Serum Vitamin D level in children with cystic fibrosis and its correlation with frequency of respiratory infections, respiratory severity score, and pulmonary function (#144). Pediatr Pulmonol 2011;46(S34):261.
[12] McPhail GL, Fenchel M, VanDyke R, et al. Vitamin D status is not associated with the rate of decline in FEV 1% in children with cystic fibrosis (#83). Pediatr Pulmonol 2011;46(S34):240.
[13] Mallowe A, Michel S, Kelly A. Vitamin D supplementation and serum vitamin D in pediatric CF patients (#496). Pediatr Pulmonol 2011;46 (S34):393.
[14] McCauley LA, Thomas W, Laguna TA, et al. Vitamin D deficiency is associated with pulmonary exacerbations in children with cystic fibrosis. Ann Am Thorac Soc 2014;11(2):198–204.
[15] Pincikova T, Paquin-Proulx D, Sandberg JK, et al. Clinical impact of vitamin D treatment in cystic fibrosis: a pilot randomized, controlled trial. Eur J Clin Nutr 2017;71(2):203–5. https://doi.org/10.1038/ejcn.2016.259 [published Online First: Epub Date].
[16] Sexauer WP, Hadeh A, Ohman-Strickland PA, et al. Vitamin D deficiency is associated with pulmonary dysfunction in cystic fibrosis. J Cyst Fibros 2015;14(4):497–506. https://doi.org/10.1016/j.jcf.2014.12.006 [published Online First: Epub Date].
[17] Agbedia OO, Saldanha IJ, Donath E, et al. Correlation between vitamin D and lung function in cystic fibrosis: results of a systematic review (#517). Pediatr Pulmonol 2011;46(S34):79–105.
[18] Simoneau T, Bazzaz O, Sawicki GS, et al. Vitamin D status in children with cystic fibrosis: associations with inflammation and bacterial colonization. Ann Am Thorac Soc 2014;11(2):205–10.
[19] Hilliard TN, Regamey N, Shute JK, et al. Airway remodelling in children with cystic fibrosis. Thorax 2007;62(12):1074–80.
[20] Holick MF. Vitamin D deficiency. N Engl J Med 2007;357(3):266–81. https://doi.org/10.1056/NEJMra070553 [published Online First: Epub Date].
[21] Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. The Lancet 1998;351(9105):805–6.
[22] Tangpricha V, Kelly A, Stephenson A, et al. An update on the screening, diagnosis, management, and treatment of vitamin D deficiency in individuals with cystic fibrosis: evidence-based recommendations from the Cystic Fibrosis Foundation. J Clin Endocrinol Metab 2012;97(4): 1082–93.
[23] Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96(7):1911–30.
[25] McNally JD, Leis K, Matheson LA, et al. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatr Pulmonol 2009;44(10):981–8.
[26] Chalmers JD, McHugh BJ, Docherty C, et al. Vitamin-D deficiency is associated with chronic bacterial colonisation and disease severity in bronchiectasis. Thorax 2013;68(1):39–47.
[27] Ginde AA, Mansbach JM, Camargo CA, et al. Association between serum 25-hydroxyvitamin d level and upper respiratory tract infection in the third national health and nutrition examination survey. Arch Intern Med 2009; 169(4):384–90. https://doi.org/10.1001/archinternmed.2008.560 [published Online First: Epub Date].
[28] Gudas LJ, Koocher GP, Wypij DAVI. Perceptions of medical compliance in children and adolescents with cystic fibrosis. J Dev Behav Pediatr 1991; 12(4).
[29] Gilchrist FJ, Salamat S, Clayton S, et al. Bronchoalveolar lavage in children with cystic fibrosis: how many lobes should be sampled? Arch Dis Child 2011;96(3):215–7. https://doi.org/10.1136/adc.2009.177618 [published Online First: Epub Date].
[30] Douglas TA, Park J, Ranganathan S, et al. Vitamin D deficiency is associated with increased risk of lower respiratory tract infection with S aureus among infants & pre-school children with CF. Pediatr Pulmonol 2012;47(S35):316–7.
[31] Grossmann RE, Zughaier SM, Liu S, et al. Impact of vitamin D supplementation on markers of inflammation in adults with cystic fibrosis hospitalized for a pulmonary exacerbation. Eur J Clin Nutr 2012;66(9): 1072–4.
[32] Pincikova T, Svedin E, Domsgen E, et al. Cystic fibrosis bronchial epithelial cells have impaired ability to activate vitamin D. Acta Paediatr 2016;105(7):851–3. https://doi.org/10.1111/apa.13361 [published Online First: Epub Date].

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