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High-dose vitamin D after lung transplantation: A randomized trial

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KEYWORDS:
chronic lung allograft dysfunction;
lung transplantation;
outcome;
prevention;
vitamin D

BACKGROUND: Vitamin D may have innate immunomodulatory functions with potentially beneficial therapeutic effects in lung transplant recipients.

METHODS: This was a single-center, double blind, randomized, placebo-controlled, prevention trial of once-monthly oral vitamin D (cholecalciferol; 100,000 IU, $n = 44$) vs placebo ($n = 43$) during 2 years in adult lung transplant recipients enrolled from October 2010 to August 2013. Primary outcome was prevalence of chronic lung allograft dysfunction (CLAD) 3 years after transplantation. Secondary outcomes included overall survival, prevalence of acute rejection, lymphocytic bronchiolitis and infection, lung function, pulmonary and systemic inflammation, and bone mineral density.

RESULTS: All included patients underwent bilateral lung transplantation and were mostly middle-aged men with prior smoking-related emphysema. Levels of 25-hydroxy vitamin D after 1 year ($p < .001$) and 2 years ($p < .001$) were significantly higher in the vitamin D group compared with the placebo group. No difference was observed for CLAD prevalence ($p = 0.7$) or CLAD-free survival between both groups ($p = 0.7$). Secondary outcomes were overall comparable between both groups (all $p > 0.05$).

CONCLUSIONS: Once-monthly oral vitamin D supplementation after lung transplantation fails to demonstrate a significant difference in CLAD prevalence, innate immunomodulatory, or a beneficial clinical effect compared with placebo.

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Vitamin D is a fat-soluble vitamin obtained by diet or by conversion of 7-dehydrocholesterol to cholecalciferol via ultraviolet light B in the skin. To become metabolically active, cholecalciferol requires dihydroxylation. The first hydroxylation occurs in the liver, producing 25-hydroxy vitamin D (25[OH]D) or calcidiol, which is the stored form

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of vitamin D. Calcidiol has a half-life of ~3 weeks, and its systemic status is reliably indicated by serum 25(OH)D levels. Calcidiol is next converted by CYP27B1 (1α-hydroxylase) into biologically active $1,25(OH)_2D$, also called calcitriol, which mainly occurs in the kidneys but also in airway epithelial cells, alveolar macrophages, and neutrophils, expressing α1-hydroxilase and the vitamin D receptor (VDR).[1] $1,25(OH)_2D$ has an important role in calcium homeostasis but also demonstrates various biologic functions by binding to VDR, subsequently regulating gene expression and exerting endocrine, paracrine, and autocrine functions.[1]

There is no universal consensus on optimal 25(OH)D serum levels, although sufficient levels (30–50 ng/ml) are required for optimal immune and respiratory effects. Therefore, levels <30 ng/ml are considered insufficient, and a level of <20 ng/ml is considered to reflect severe vitamin D deficiency.[2,3]

$1,25(OH)_2D$ demonstrates anti-inflammatory effects in the lungs due to local inhibition of nuclear factor-κB and mitogen-activated protein kinase activity, thus attenuating secretion of inflammatory cytokines and chemokines, such as interleukin (IL)-1β, IL-6, and IL-8, and influx of inflammatory cells. Next to this, $1,25(OH)_2D$ reduces oxidative stress by inhibiting anti-protease activity and acting on nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional regulator of most antioxidant genes.[4]

$1,25(OH)_2D$ has anti-infectious properties by increasing maturation and proliferation of monocytes into macrophages and by transcriptional upregulation of cathelicidin and defensin-β2 in airway epithelial cells, pulmonary macrophages, and neutrophils,[4] which suppress inflammation and are effective in microbial killing, including fungi and *Pseudomonas* species.[5] In addition, $1,25(OH)_2D$ is involved in tissue remodeling, demonstrating inhibitory effects on the expression of matrix metalloproteinases in airway smooth muscle cells, monocytes, and alveolar macrophages, on proliferation and activation of bronchial airway muscle cells and fibroblasts, on extracellular matrix deposition by fibroblasts, and on epithelial–mesenchymal transition.[6,7] Finally, $1,25(OH)_2D$ inhibits proliferation and differentiation of B-cells, suppresses secretion of pro-inflammatory cytokines by cluster of differentiation $(CD)4^+$ T cells, inhibits T-helper (Th)-cells, such as Th1, Th9, Th17, and possibly also Th2, increases development of regulatory T cells (Tregs), and promotes a tolerogenic phenotype of dendritic cells.[1,4,8]

Given these broad immunobiologic effects, vitamin D supplementation has been studied in asthma, chronic obstructive pulmonary disease (COPD), and tuberculosis, demonstrating improved pulmonary function and reduced airway remodeling and disease exacerbations.[1] Also in lung transplantation (LTx), low 25(OH)D levels ($<$ 30 ng/ml) were associated with increased acute rejection and infection rates, lower forced expiratory volume in 1 second (FEV_1), and worse survival after LTx.[9–11] Interestingly, in a rat orthotopic LTx model, $1,25(OH)_2D$ substitution attenuated acute peri-vascular (A-grade) and peri-bronchiolar (B-grade) cellular rejection.[12] These findings are important given that asymptomatic vitamin D deficiency is present in 26% to 47% of LTx recipients.[9–11]

Although plausible immunologic benefits may be associated with vitamin D supplementation, this therapy has not translated into definitive and concrete clinical benefits in respiratory disorders or after LTx in particular. In view of the significant morbidity and mortality associated with chronic lung allograft dysfunction (CLAD) after LTx, we therefore investigated the possible beneficial effects of high-dose vitamin D supplementation on CLAD prevalence and CLAD-free survival in a randomized controlled prevention trial. Also assessed were secondary effects on overall survival, lung function, acute rejection, lymphocytic bronchiolitis and infection, pulmonary and systemic inflammation, and bone mineral density.

Methods

Trial design

An investigator-driven, double-blind, randomized placebo-controlled trial of oral vitamin D vs placebo, given in addition to standard of care treatment, was conducted at the University Hospitals Leuven, Belgium. Eligible patients were included at hospital discharge after LTx by their treating transplant physician (L.J.D., G.M.V.). After written informed consent, patients were randomly assigned to receive oral vitamin D or placebo. Study groups were generated through permuted-block randomization using a 1:1 ratio by the University's Hospital Experimental Pharmacy (http://www.randomization.com). All participants, nurses, and treating physicians were blinded to group assignment during the study treatment and later follow-up. Evaluation of outcomes was performed after completion of the 3-year post-LTx follow-up of the last included study patient in a blinded manner by investigators of the Leuven University Lab of Respiratory Diseases (R.V., D.R., S.V.). The Hospital's Clinical Trial Unit and Institutional Review Board (S52577) approved this study, which was registered at Clinicaltrial.gov (Clinicaltrial.gov identifier: NTC01212406).

Participants

Single-lung, bilateral-lung, or heart-lung transplant recipients aged at least 18 years old and able to take oral drugs at hospital discharge after LTx were eligible for inclusion. Patients were not included if they had died within 30 days after LTx, had stayed more than 30 days in the intensive care unit (ICU), had important bronchial anastomotic complications (stenosis or dehiscence) at discharge, received a transplant for cystic fibrosis (CF), underwent repeat LTx, multiorgan transplantation, or previously underwent solid-organ or bone marrow transplantation. Also, excluded from the current trial were patients who had been included in a clinical trial with ex vivo lung perfusion (information about preceding inclusion in ex vivo lung perfusion study was only revealed to the treating physician upon discharge after signed informed consent for the current study).

All clinical, biometric, and histologic data acquired during the study and later follow-up were automatically stored in patients' individual electronic medical files and clinical and pathologic databases of the University Hospital, from which they were obtained later for further analyses of outcome parameters and saved in a separate anonymized file on an access-protected server of the

University Lab of Respiratory Diseases conforming to our local Biobank policy (S51577).

Interventions

Vitamin D (D-Cure Forte; cholecalciferol, 100,000 IU, 4 ml, orally, once monthly) was provided by Laboratoires S.M.D (Brussels, Belgium). The placebo was arachide oil (4 ml, orally, once monthly; Fagron, Rotterdam, The Netherlands). The vitamin D and placebo were stored and delivered by the University's Hospital Experimental Pharmacy in blinded, sequentially numbered syringes (ExactaMed 10 ml, 1610 Amber; Baxter) for oral intake by each included patient. Study medication was provided at routine outpatient follow-up visits or during hospital admissions by a dedicated nurse, who also verified compliance and adverse events at each contact.

Patients were instructed to continue study treatment for 2 years. Study unblinding was only performed after completion of the 3-year post-transplant follow-up of the last included patient. Study treatment was only to be stopped in case of withdrawal of consent or in case of serious adverse events, re-LTx, or death. In case of suspected or confirmed CLAD, study treatment was continued, and standard rescue therapy was initiated as described in the Supplementary Materials (available online at www.jhltonline.org).

Outcomes

Primary outcome comprised CLAD prevalence at 3 years post-LTx, including bronchiolitis obliterans syndrome (BOS) and restrictive CLAD, also called restrictive allograft syndrome (RAS), according to the changing definitions of chronic lung allograft rejection/dysfunction since the study was initially designed,[13] evolving experience regarding beneficial effects of azithromycin in suspected BOS/CLAD (i.e., azithromycin reversible allograft dysfunction),[14,15] and liberal use of azithromycin in patients with suspected BOS/CLAD in our cohort since 2011.[16–18]

Secondary outcomes included overall survival and FEV_1 during the first 3 years after LTx; prevalence of acute rejection, lymphocytic bronchiolitis, and pulmonary infection; bronchoalveolar lavage (BAL) cellularity and cytokine/protein levels; and as blood C-reactive protein levels during the first 2 years after LTx (i.e., during standardized surveillance follow-up until 2 years). Bone mineral density was also assessed during the first 3 years after LTx.

Serious adverse events were defined as serious allergic reactions, including skin reactions (rash, urticarial, Stevens-Johnson syndrome), angioneurotic edema and anaphylaxis, or hypercalcemia >3.5 mmol/L during the study.

Standard of care management

Standard immunosuppressive regimen, infectious prophylaxis, and CLAD diagnosis were previously described[15–20] and are summarized in the Supplementary Materials (online), as is assessment of 25(OH)D serum levels[21] and of bone mineral density.

After LTx, all patients in both study groups received daily vitamin D supplements for osteoporosis prevention/treatment (2,500 mg calcium carbonate and 880 IU cholecalciferol concentrate), which was restarted before discharge (if initiated before LTx) or de novo initiated before discharge according to standard of care practice. Bisphosphonates were restarted at discharge (if initiated before transplant) or de novo initiated during later follow-up in case of severe osteoporosis according to local reimbursement criteria (T-score < -2.5 and/or vertebral insufficiency fracture of $\geq 25\%$).

Statistical analysis

Assuming a 30% BOS prevalence at 2 years in our population,[17,18] a type I error rate of $\alpha = 0.05$, a type II error rate of $\beta = 0.20$, and an absolute reduction in BOS (stage $\geq$ 1) of 25% after 2 years, sample size calculation estimated that at least 35 patients per group, or 70 patients total, had to be included.

Incorporating possible patient exclusions after initial randomization (15%) and dropouts during later follow-up (15%), 50 patients per group, or a total of 100 patients, were foreseen for study enrollment. Assuming a mean annual number of 52 transplantations during the preceding years and exclusion criteria precluding 30% of our patients for study inclusion, a 3-year enrollment period was anticipated to complete patient inclusion.

All patients initiated on study medication were included in intention-to-treat outcome analyses using GraphPad Prism 5a software (GraphPad Software Inc., La Jolla, CA). Results are expressed as mean ± standard deviation or median (interquartile range) wherever appropriate. Group means were compared using unpaired, 2-tailed *t*-test, Mann-Whitney test, or Wilcoxon signed rank test, or one-way analysis of variance (either repeated-measures analysis of variance with Bonferroni post-test, or Kruskal-Wallis with Dunn post- test), respectively, depending on normality distribution and repeated measures. Fisher's exact test was used to compare proportions. Kaplan-Meier survival curves and log-rank analysis was used for time-to-event analysis regarding CLAD and overall survival. For the end point of CLAD, survival times were censored at death or at study discontinuation if these preceded CLAD or else at 3 years after LTx. For the end point of death, survival times were censored at death or at 3 years after LTx. All *p*-values are 2-tailed, and $p < .05$ was considered statistically significant.

Results

Patients' characteristics

The current study randomized 100 patients from October 2010 to August 2013. Study flow chart, patient inclusion (placebo, $n = 43$; azithromycin, $n = 44$) and reasons for exclusion, are summarized in Figure 1. Baseline characteristics were similar in both groups (Table 1). All included patients underwent bilateral LTx and were mostly middle-aged men with prior smoking-related emphysema. Before LTx, most patients received vitamin D supplements for osteoporosis, but proportionally no difference was present between both groups: 56% in placebo vs 61% in the vitamin D group ($p = 0.7$). Levels of 25(OH)D at LTx were comparable, but generally insufficient (i.e., <30 ng/ml) in the placebo (24.3 ± 6.6 ng/ml) and vitamin D (24.9 ± 5.5 ng/ml) groups ($p = 0.6$). Overall, 25.3% of all included patients were severely deficient in vitamin D (<20 ng/ml; placebo vs vitamin D: $p = 0.1$).

The baseline immunosuppressive regimen was similar in both groups, and at 3 years after LTx, azithromycin had been initiated in 56% of the placebo group and in 73% of the vitamin D group ($p = 0.1$). Time from LTx to start of azithromycin was comparable between both groups: 187 (91-550) days in the placebo group vs 207 (89-649) days in the vitamin D group ($p = 1.0$).

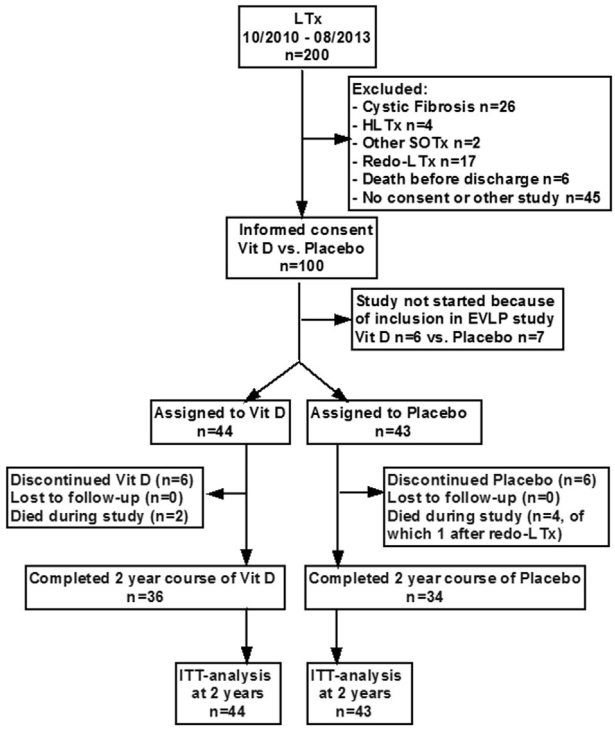

Figure 1 Flow chart shows study enrollment and inclusion in the intention-to-treat analysis. EVLP, ex vivo lung transplantation; HLTx, heart-lung transplantation; ITT, intention-to-treat; LTx, lung transplantation; SOTx, solid-organ transplantation; Vit, vitamin.

Study treatment duration was comparable between both groups ($p = 0.8$). Study drug discontinuation occurred in 6 patients (13.9%) assigned to placebo and in 6 patients (13.6%) assigned to vitamin D ($p = 1.0$; Table 2). Discontinuation was almost exclusively due to withdrawal of consent by the patients. One patient in the vitamin D group stopped study treatment because of intolerance (nausea) after the second monthly dose. Overall, 34 patients (79.1%) assigned to placebo and 36 patients (81.8%) assigned to the vitamin D group completed the 2-year study drug treatment period. Both in placebo and in vitamin D groups, 25(OH)D levels significantly increased at 1 and 2 years after LTx compared with levels at LTx (both $p <$ .0001, respectively), but this increase was more pronounced in the vitamin D group than in the placebo group (Figure 2). At 3 years after LTx (i.e., after 1 year wash-out of study treatment), 25(OH)D levels were overall higher compared with levels at LTx but, as expected, were comparable between both groups ($p = 0.7$).

Primary outcome

CLAD prevalence at 3 years after LTx was similar in both groups: 6 in placebo (14%) vs 5 in vitamin D (11%; $p = 0.7$). The proportion of BOS and RAS was similar in both groups: 3 of 6 BOS and 3 of 6 RAS in the placebo group vs 3 of 5 BOS and 2 of 5 RAS in the vitamin D group. CLAD-free survival was comparable between both groups ($p = 0.7$; Figure 3).

Secondary outcomes

Overall survival

Mortality at 3 years after LTx was similar in both groups: 5 in placebo (12%) vs 3 in vitamin D (7%; $p = 0.5$). Kaplan-Meier survival estimates were comparable between both groups ($p = 0.4$; Figure 4). In the placebo group, 2 patients died of CLAD (both RAS, 1 of whom died of multisystem organ failure after re-LTx), 2 died of fungal infection, and 1 died of thrombotic microangiopathy with multisystem organ failure. In the vitamin D group, 2 patients also died of progressive CLAD (both RAS), and 1 died of throat cancer.

Pulmonary function and exercise capacity

No differences were seen in FEV_1 (% predicted) between the placebo and vitamin D groups at study inclusion or after 1, 2, or 3 years (all $p > 0.05$; Tables 1 and 2). The best post-operative FEV_1 during the study was also comparable ($p = 0.4$). Nevertheless, a weak correlation was observed between 25(OH) D levels at 1 or 2 years ($r^2 = 0.048$, $p = 0.053$) and the best post-operative FEV_1 ($r^2 = 0.052$, $p = 0.047$).

No differences were seen in the 6-minute walk distance between the placebo and vitamin D groups at study inclusion or after 1, 2, or 3 years (all $p > 0.05$; Tables 1 and 2).

Acute rejection, lymphocytic bronchiolitis, and infection rates

The prevalence of acute rejection (AR; $p = 1.0$), severe AR (grade $\geq$ A2; $p = 0.8$), lymphocytic bronchiolitis ($p = 0.9$), severe lymphocytic bronchiolitis (grade $\geq$ B1R; $p = 0.4$), or respiratory infection ($p = 0.9$) was comparable between both groups during the 2-year treatment period. Sub-analysis of infections caused by *Aspergillus* spp and *Pseudomonas aeruginosa* revealed no differences between both groups ($p = 0.2$ and $p = 1.0$, respectively; Table 2).

Pulmonary and systemic inflammation

BAL total and differential cell counts, IL-6 and IL-8 protein levels, and blood C-reactive protein levels were comparable between both groups during the 2-year treatment period (all $p > 0.05$; Supplementary Table S1, online).

Bone mineral density

The proportion of patients treated with bisphosphonates during the first 3 years after LTx was similar between both groups: 33% in placebo vs 39% in vitamin D ($p = 0.1$; Table 2). In only 8% of included patients was the bone mineral density within normal reference ranges before LTx (9% in placebo and 7% in vitamin D, $p = 0.7$), and notably, 44% of patients had osteoporosis before LTx (42% in placebo and 45% in vitamin D, $p = 0.8$). Overall, bone

Table 1 Patient Demographics

Variable[a]	Placebo ($n = 43$)	Vitamin D ($n = 44$)	p-value
Age at LTx, years	57.3 ± 4.7	55.8 ± 6.6	0.4
Sex			0.8
Male	23	25	
Female	20	19	
Indication for LTx			0.2
Emphysema	32 (74)	30 (68)	
Pulmonary fibrosis	8 (19)	10 (23)	
Pulmonary arterial hypertension	3 (7)	1 (2)	
Other	0	3 (7)	
BMI at LTx, kg/m^2	22.9 ± 4.3	22.5 ± 3.6	0.8
Pack-years smoked before LTx, No.	30 (20–40)	30 (3.5–37.8)	0.2
Vitamin D supplement before LTx	24 (56)	27 (61)	0.7
Double or bilateral LTx	43 (100)	44 (100)	NA
Ischemic times, min			
First lung	257.5 (224.3–299.8)	244.5 (209.5–296.3	0.4
Second lung	411.5 (368.3–452.0)	397.0 (349.8–441.3)	0.2
ECMO use	6 (14)	7 (16)	1
DBD donor	35	32	
DCD donor	8	12	0.5
CMV mismatch (D+/R–)	8 (19)	5 (11)	0.4
Time from LTx to			
Hospital discharge, days	33 (28–44)	32 (28–36)	0.4
Start of study, days	34 (29–44)	33 (29–40)	0.4
Stop of study, days	737 (726–762)	737 (725–754)	0.8
Total time of follow-up, days	1,382 ± 448.7	1,438 ± 354.2	1
Initial IS regimen at discharge			
Calcineurin inhibitor			0.4
Tacrolimus (FK506)	43	43	
Cyclosporine A	3	1	
None	0	0	
Cell cycle inhibitor			0.1
Mycophenolate mofetil	35	37	
Azathioprine	8	4	
None	0	0	
Steroids	43	44	NA
At the start of the study			
FEV_1, liters	2.05 ± 0.73	2.19 ± 0.69	0.3
FEV_1, % predicted	67.3 ± 16.7	69.1 ± 17.3	0.7
6MWD, m	245.1 ± 134.8	256.9 ± 123.3	0.6
Serum creatinine, mg/dl	1.05 ± 0.40	1.01 ± 0.40	0.6
Serum calcium, mmol/liter	2.29 ± 0.11	2.28 ± 0.11	0.6
25(OH)D levels, ng/ml	24.3 ± 6.6	24.9 ± 5.5	0.6
Azithromycin at 3 years post-LTx	24 (56)	32 (73)	0.1
Time from LTx to start of azithromycin, d	187 (91–550)	207 (89–649)	1

BMI, body mass index; CMV, cytomegalovirus; D, donor; DBD, donor after brain death; DCD, donor after cardiac death; ECMO, extracorporeal membrane oxygenation; F, female; FEV_1, forced expiratory volume in 1 second; IS, immunosuppressive; LTx, lung transplantation; M, male; NA, not applicable; R; recipient; 6MWD, 6-minute walking distance; 25(OH)D, 25-hydroxy vitamin D.

[a]Continuous data are presented as mean ± standard deviation or median (interquartile range) and categoric data are presented as total values (%).

mineral density before LTx was comparable between the placebo and vitamin D groups for the spine ($p = 0.5$) and femur ($p = 0.5$). Bone mineral density remained comparable between both groups during the 2-year treatment period (all $p > 0.05$). At 3 years after LTx, patients previously assigned to receive vitamin D demonstrated lower spinal ($p = 0.04$) but similar femoral ($p = 0.2$) T-scores compared with the placebo group.

Side effects and adverse events

No allergic reactions attributable to study treatment were reported. No patients developed hypercalcemia (>3.5 mmol/L). Urolithiasis occurred in 1 patient assigned to vitamin D during the 2-year treatment period. The patient was treated with temporary urethral double J stenting and shock wave lithotripsy (without unbinding of study drug).

Table 2 Study Outcomes

Outcome[a]	Placebo ($n = 43$)	Vitamin D ($n = 44$)	p-value
Primary outcome			
CLAD at 3 years	6 (14)	5 (11)	0.7
BOS	3	3	
RAS	3	2	
Secondary outcomes			
Death at 3 years	5 (12)	3 (7)	0.5
Cause of death			
Infection	2	0	NA
CLAD (all RAS)	2	2	NA
Cancer	0	1	
Other	1	0	
Redo LTx, *n* (%)	1 (2)	0	
FEV_1, % predicted			
At 1 year	86.1 ± 16.3	89.1 ± 22.9	0.3
At 2 years	90.9 ± 18.5	89.4 ± 23.3	0.8
At 3 years	93.0 ± 21.0	97.2 ± 25.2	0.4
Best FEV_1 until 3years, % predicted	95.4 ± 17.2	98.9 ± 22.2	0.4
6-MWD at 1 year, m	497.0 ± 98.1	493.2 ± 125.7	0.9
6-MWD at 2 years, m	525.4 ± 94.1	543.4 ± 104.5	0.5
Outcomes at 2 years			
AR, No.	0.39 ± 0.79	0.36 ± 0.65	1
AR grade ≥ 2 , No.	0.14 ± 0.35	0.15 ± 0.37	0.8
LB at 2 years, No.	0.64 ± 0.88	0.61 ± 0.81	0.9
LB grade ≥ 2, No.	0.16 ± 0.37	0.23 ± 0.42	0.5
LB grade > B1R, No.	0.046 ± 0.21	0.090 ± 0.29	0.4
Infections, No.	0.44 ± 0.77	0.57 ± 1.07	0.6
Aspergillus	0.070 ± 0.26	0.16 ± 0.37	0.2
Pseudomonas	0.024 ± 0.15	0.023 ± 0.15	1
Calcium, mmol/liter			
At 0 year	2.30 ± 0.11	2.28 ± 0.11	0.6
At 1 year	2.35 ± 0.10	2.34 ± 0.11	0.1
At 2 years	2.37 ± 0.09[b]	2.34 ± 0.08	0.1
At 3 years	2.39 ± 0.13[c]	2.35 ± 0.10[b]	0.1
ANOVA p-value	0.0018	0.011	
Creatinine, mg/dl			
At 0 years	1.05 ± 0.40	1.01 ± 0.40	0.6
At 1 year	1.36 ± 0.33[d]	1.33 ± 0.41[d]	0.5
At 2 years	1.44 ± 0.38[d]	1.38 ± 0.40[d]	0.4
At 3 years	1.49 ± 0.46[d]	1.54 ± 1.10[d]	0.3
ANOVA p-value	<0.0001	<0.0001	
Bone density spine, T-score			
At 0 years	−1.7 ± 1.6	−2.1 ± 1.3	0.5
At 1 year	−1.8 ± 1.2	−2.1 ± 1.1	0.5
At 2 years	−1.5 ± 1.2	−1.9 ± 1.1	0.2
At 3 years	−1.3 ± 1.2	−1.9 ± 1.1	0.04
Bone density femur, T-score			
At 0 years	−1.8 ± 1.2	−2.0 ± 1.0	0.5
At 1 year	−2.1 ± 0.9	−2.3 ± 0.7	0.3
At 2 years	−2.1 ± 0.9	−2.4 ± 0.7	0.06
At 3 years	−2.1 ± 0.9	−2.3 ± 0.7	0.2
Drop-outs			
Withdrawal of consent	5	5	1
Intolerance of patient (nausea)	0	1	1
Stop by treating physician	1 (redo LTx)	0	
Adverse events			
Urolithiasis	0	1	
Hypercalcemia (>3.5 mmol/L)	0	0	

ANOVA, analysis of variance; AR, acute rejection; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; FEV_1, forced expiratory volume in 1 second; LB, lymphocytic bronchiolitis; LTx, lung transplant; NA, not applicable; RAS, restrictive allograft syndrome; 0 years: at start of study; 6-MWD, 6-minute walking distance.

[a]Continous data are represented as mean ± standard deviation and categoric data as total values (percentage).

[b]$p < .05$,

[c]$p < .01$

[d]$p < .001$ vs 0 years.

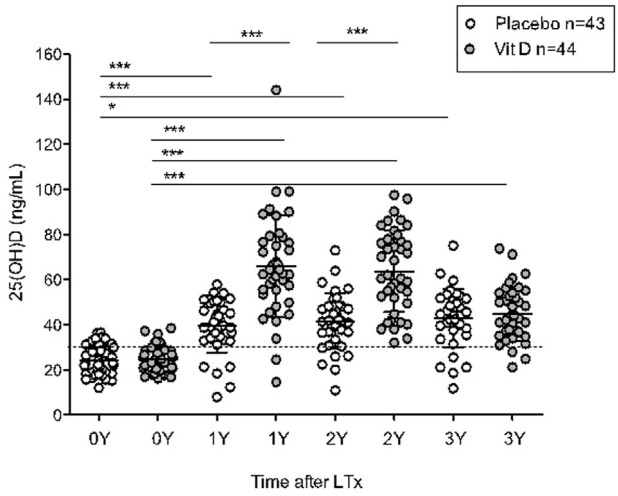

Figure 2 Serum 25-hydroxy vitamin (Vit) D (25[OH]D) levels in patients assigned to placebo ($n = 43$) or vitamin D ($n = 44$) at start of study (0Y), 1 year (1Y), 2 years (2Y), and 3 years (3Y) after lung transplantation (LTx), respectively.*$p < .05$, **$p < .01$, ***$p < .001$.

All patients, except 1 (nausea, dropout), tolerated the study medication without any reported side effects. Blood calcemia and creatinine levels were comparable between the groups at study inclusion and after 1, 2, or 3 years, although a significant increase was noticed in both groups for both parameters over time after LTx (Table 2).

Discussion

This randomized trial investigated a possible beneficial effect of prophylactic treatment with high doses of vitamin D after LTx. However, we could not demonstrate a reduction in CLAD prevalence or improved CLAD-free survival compared with placebo. Also, no benefits were seen regarding overall survival, FEV_1, acute rejection, lymphocytic bronchiolitis, respiratory infections, pulmonary and systemic inflammation, or bone mineral density.

These negative results may seem unexpected, given the broad immunobiologic effects attributed to vitamin D,[4–12] as was recently corroborated by a multicenter trial of prophylactic vitamin D (1,000 to 5,000 IU/day for 105 days) after allogeneic stem cell transplantation.[22] A significantly lower incidence of chronic graft-vs-host-disease among patients receiving vitamin D was seen, which was attributed to attenuated immune responses, including decreased number of B-cells and naïve $CD8^+$ T cells, and lower expression of CD40L.[22] However, this trial was performed in a completely different clinical setting compared with our study and included no patients with pulmonary involvement of chronic graft-vs-host-disease.

In line with our current negative results, various other randomized trials in the field of lung diseases were also unable to demonstrate a positive effect of vitamin D supplementation on their respective primary outcomes. For instance, high-dose vitamin D (250,000 or 500,000 IU, 5 days) safely increased 25(OH)D levels in ICU patients yet without altering important clinical outcomes such as ICU- and hospital-acquired infections, occurrence of organ dysfunction, or duration of mechanical ventilation.[23] Also, in a pulmonary tuberculosis cohort, vitamin D deficiency was safely corrected by supplements (50,000 IU thrice weekly for 8 weeks, thereafter 50,000 IU every other week for 8 weeks), yet without improving sputum *Mycobacterium tuberculosis* clearance.[24]

Vitamin D supplementation (250,000 IU once) reduced some markers of systemic inflammation in adults with CF hospitalized for a pulmonary exacerbation,[25] yet again without significantly improving clinical outcomes of lung function or antibiotic therapy–free days.[26] In moderate to severe COPD, high-dose, long-term vitamin D (100,000 IU every 4 weeks for 1 year) failed to reduce the incidence of exacerbations; also unaffected were the secondary outcomes of FEV_1, quality of life, and death.[27] Moreover, prospective analysis demonstrated that 25(OH)D levels were not associated with mortality in moderate to severe COPD.[28]

Similarly, vitamin D (100,000 IU once, then 4,000 IU/day for 28 weeks) did not reduce the rate of first treatment failure or exacerbation in persistent adult asthma with concurrent vitamin D insufficiency.[29] Prospective evaluation demonstrated that 25(OH)D levels did not influence later development of asthma or allergy and were not associated with lung function in a large cohort of Danish adults.[30] Finally, prenatal vitamin D supplementation in pregnancy (either 200,000 IU once or 2,400 IU/day during the third trimester) was not associated with decreased infancy wheezing in offspring.[31,32]

In general, one can conclude that there is at least an important discrepancy between in vitro and preliminary clinical results of vitamin D in lung disorders and the results obtained from randomized trials. This highlights that further research is needed to elucidate the pathophysiologic mechanisms involving vitamin D, possible interfering factors, precise dosing, and probable benefits of vitamin D on relevant clinical outcomes in respiratory disorders. Altogether, the findings from various randomized trials currently do not support therapeutic or preventive vitamin D supplementation in patients with lung disorders.

Nevertheless, our study is important because it was performed in LTx, a field in which there is an urgent need for preventive studies regarding CLAD, which remains the major long-term complication after LTx complication.[33] A probable explanation for the lack of any positive results in our trial may be the high prevalence of azithromycin use in our population (i.e., overall 64% at 3 years). Indeed, azithromycin is a very potent immunomodulatory and anti-inflammatory drug, positively affecting early and long-term post-LTx outcomes such as lymphocytic airway inflammation,[16] CLAD,[18,34] and overall survival after CLAD onset.[17,35] Moreover, many pathways similar to those attenuated by vitamin D, such as inhibition of nuclear factor-κB and mitogen-activated protein kinase activity, oxidative stress, and remodelling, are modulated by azithromycin.[15] Therefore, that no beneficial effects were seen regarding CLAD, infections, or inflammatory markers between both groups in our study may not be surprising. Interestingly, annual CLAD incidence during the initial

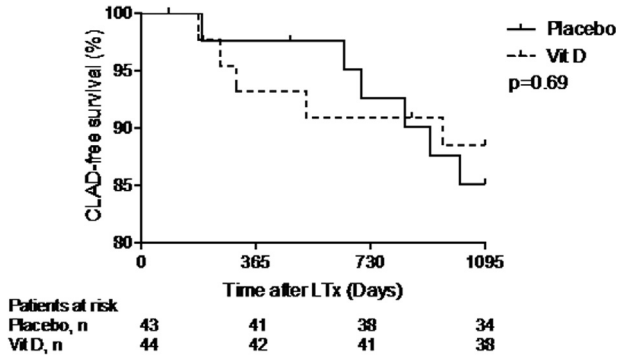

Figure 3 Kaplan-Meier survival curves show chronic lung allograft dysfunction–free survival after lung transplantation (LTx) in patients assigned to placebo ($n = 43$) or vitamin (Vit) D ($n = 44$). Tick represents censored subjects.

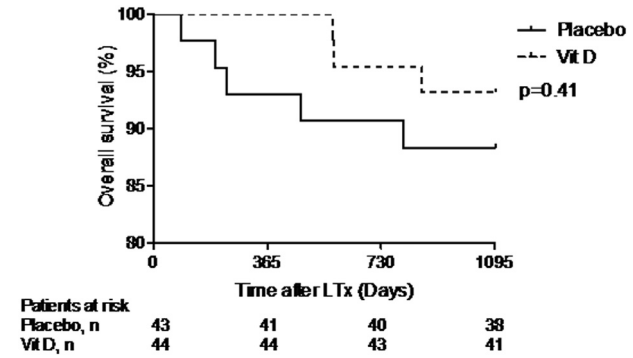

Figure 4 Kaplan-Meier survival curves show overall survival after lung transplantation (LTx) in patients assigned to placebo ($n = 43$) or vitamin (Vit) D ($n = 44$).

3 years post-LTx in the current study was only 4.2% (i.e., overall 12.6% CLAD prevalence at 3 years in the entire study cohort), which approximates the 3% annual CLAD incidence previously documented in patients preemptively treated with azithromycin (i.e., 9% CLAD-prevalence at 3 years in patients assigned to the azithromycin arm).[34] These results are encouraging and nonetheless considerably better compared with the reported CLAD prevalence in international registries.[33]

Our current study confirms previous findings of pre-existing vitamin D deficiency in an important proportion of LTx candidates, as is evidenced by average 25(OH)D levels of <30 ng/ml and overall some 25% of candidates demonstrating severe vitamin D deficiency (<20 ng/ml) at LTx. Moreover, osteopenia (overall 92% in our study) and osteoporosis (44% in our study) are very common features in LTx candidates, most likely resulting from a sedentary life-style and prior long-term treatment with corticosteroids. Although high-dose vitamin D supplementation increased serum vitamin D levels more effectively than standard supplementation with low-dose vitamin D (880 IU/day), the latter also effectively increased post-LTx vitamin D levels to a normal range of 30 to 50 ng/ml. No beneficial effects were seen on bone mineral density with high-dose vitamin D supplementation in the current study, which should thus not be advocated as a therapy for osteoporosis or as prevention of osteoporotic fractures after LTx. However, long-term, high-dose vitamin D supplementation seems to be safe regarding hypercalcemia and urolithiasis, which are its most important adverse effects, because no significant increase was observed in patients assigned to vitamin D.

Inevitable limitations of this trial are inherent to its design, single-center approach, specific patient selection criteria, and number of included patients. Although follow-up time was appropriate to assess long-term post-LTx outcomes, the lower-than-expected event rates substantially reduced the power of our study to detect a significant difference with the current sample size.

Another important issue may be that we excluded CF patients. Most of these patients require higher oral vitamin D doses to achieve adequate serum 25(OH)D levels (i.e., CF patients in our center receive at least 1,000 IU vitamin D daily) because gastrointestinal absorbance of oral vitamin D supplements in many of these patients is variable as a result of underlying pancreatic insufficiency and malabsorption of fat-soluble vitamins. Therefore, our results cannot be generalized for all LTx recipients, because CF patients comprise about 25% of all LTx recipients worldwide.[33]

Another possible bias may be adherence to the study treatment. All patients indicated they were compliant regarding study drug intake, and 25(OH)D levels in almost all patients assigned to vitamin D indeed demonstrated sufficiently high levels (>30 ng/ml). As such, only 2 patients assigned to vitamin D demonstrated suboptimal 25(OH)D levels (<30 ng/ml) at 1 year after LTx, both of which had previously stopped the study drug (dropped out after withdrawal of consent) at 2 years post-LTx; however, all patients were above this cutoff. Therefore, we do not believe that non-compliance to study treatment contributed to the negative results.

In conclusion, despite safely increasing serum 25(OH)D levels to a sufficient range, prophylactic treatment with high-dose vitamin D after LTx fails to demonstrate a significant difference in CLAD prevalence, overall survival, pulmonary function, acute rejection, lymphocytic bronchiolitis, respiratory infections, bone mineral density, and local or systemic inflammation compared with placebo.

Disclosure statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

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Supplementary data

Supplementary data are available in the online version of this article at www.jhltonline.org.

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