Dear Authors,

On behalf of the Editorial board of *Wiki J Med* I would like to thank you for your submission.

I have now performed the preliminary Editorial review of your article. Overall this is an interesting, mostly well written and methodologically very well performed article. The main strength of this work is that it is a pre-registered systematic review and meta-analysis on the use of vitamin D as an adjunct treatment for pneumonia in infants and children under five years. This is an important topic from a global health perspective. Since this is a registered meta-analysis the authors have an obligation to publish it regardless of the findings. Furthermore, the authors appear to be well versed in performing systematic reviews and meta-analyses along state-of-the-art methodologies.

The work also has several limitations (as any article does). There are already numerous systematic reviews and meta-analyses on vitamin D (more than there are RCTs). No previous meta-analysis has focused on this particular question although several have focused on the role of vitamin D in preventing respiratory tract infections, which is arguably the more logical research question. Furthermore, you will see below that I have some concerns about excluding populations with rickets. Please find these comments below, I hope you will find these useful to improve your manuscript further.

Yours sincerely,

Michaël R. Laurent, MD PhD

# Vitamin D as an adjunct for acute community-acquired pneumonia among infants and children: systematic review and meta-analyses

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**Abstract**

**Background:**

Community-acquired pneumonia (CAP) is a major cause of mortality and morbidity among infants and children particularly in low and middle income countries where malnutrition rates are very high. Vitamin D which plays a pivotal role in innate as well as adaptive immunity is a probable candidate low-cost intervention for use as an adjunct for treatment of pneumonia.

**Methods:** We searched multiple electronic databases (20 June 2016) as well as grey literature to search for randomised controlled trials (RCTs) on vitamin D being used as an adjunct in infants and children with CAP. We used the Cochrane methodology for assessing risk of bias and where adequate data was available conducted a meta-analysis using a random-effects model. We assessed overall evidence quality using the GRADE approach.

**Findings:** We screened 323 241 unique papers and identified two completed and two ongoing trials based on our inclusion criteria. The two completed trials were from India and Afghanistan. There was no significant difference in clinical cure rates (risk ratio (RR)1.01; 95% confidence interval (CI) 0.91, 1.13) in the only study which reported it and the quality was low on GRADE criteria. Data on time-to-clinical recovery of pneumonia could not be pooled due to paucity of data but there was no significant differences between vitamin D and placebo groups in both the completed studies. The pooled all-cause mortality was not significantly different between vitamin D and placebo groups (RR 1.50; 95% CI 0.25, 9.17) and this was low quality on GRADE criteria. The total duration of hospital stay was reported in one trial and that was not significant. None of the trials reported data on time-to-radiological resolution of pneumonia, requirement of additional interventions, or complications of pneumonia.

**Conclusions:** There is insufficient evidence available from RCTs to permit judgement about the use of vitamin D in infants and children with CAP. There is a greater need for more RCTs on this issue in diverse settings.

**Key Words:** Pneumonia, vitamin D, children, infants, systematic review, meta-analysis

**Met opmerkingen [ML1]:** Please provide ORCID numbers for all authors.

Secondly, is there a Wikipedia article (if any) that you think might benefit from including some of the content of this manuscript? (my guess would be that the answer is no, which by the way doesn't influence the chances of getting published in our journal at all, we also accept papers with academic merit in their own right without direct benefit to Wikimedia content).

**Met opmerkingen [ML2]:** Please mention that this meta-analysis was registered with PROSPERO in the abstract, and provide the number.

**Met opmerkingen [ML3]:** I would substract the duplicates from this count.

**Met opmerkingen [ML4]:** Please provide some basic information on these two trials e.g. trial size, average baseline vitamin D status etc. in the abstract.

**Introduction**

Worldwide, pneumonia is the leading cause of mortality among under-five children and accounts for approximately 1.3 million deaths annually [1]. Most of these deaths occur in South Asia and Africa where the prevalence of malnutrition among children is high. The consequences of pneumonia are even more severe on account of lower immunity among children with malnutrition [2]. Low-cost interventions for better management of pneumonia, particularly those which can boost the immune response, might hence contribute to decreased ~~under-five~~ mortality rates from pneumonia in children.

The role of vitamin D in immunity and subsequently in pathogenesis, treatment and prevention of human infectious diseases, particularly of the respiratory tract, has come into limelight in the last few decades, although its role in tuberculosis has been postulated for more than a century [3-6]. The vitamin D receptor (VDR) and CYP27B1, which converts vitamin D into its active form, has been found in macrophages, monocytes, dendritic cells and respiratory epithelial cells –which play a pivotal role in innate as well as adaptive ~~system~~ immune responses [7,8]. Vitamin D exerts its immunomodulatory effects by regulating the production of the antimicrobial peptides cathelicidin LL-37 and beta-defensin-2 (innate immunity) and also by regulating the TH1 cells, TH2 cells, TH17 and T regulatory cells (adaptive immunity [8,9,10]. Pathogens causing pneumonia stimulate Toll-like receptors which activates macrophages as well as up-regulates VDR and ~~CYP27A1~~ CYP27B1 and leads to the initiation of Cathelicidin LL-37 production. Cathelicidin is an antimicrobial peptide which can kill pathogens by a direct action or by binding to endotoxins or formation of ion channels resulting in loss of permeability.

Vitamin D deficiency has been linked to increased susceptibility of various infectious diseases, pneumonia and tuberculosis being the most prominent among them. The role of vitamin D in preventing infections has been studied in multiple randomised controlled trials (RCTs). A systematic review which investigated the role of vitamin D supplementation for prevention of respiratory infections indicates a protective role of vitamin D [11, 12]. Vitamin D levels ha~~s~~ve also been seen to be correlated to severity of pneumonia in children in various case-control studies [13-15]. ~~and therefore its~~ Given this background, the use of vitamin D supplements as an adjuvant for treatment of community-acquired pneumonia (CAP) might improve clinical outcomes, however this has never been systematically reviewed. The aim of ~~T~~this paper is therefore to ~~has~~ systematically review~~ed~~ the therapeutic effect of vitamin D as an adjunct ~~in~~ for the treatment of CAP ~~for~~ in infants and children.

**Methods**

We searched for published and unpublished RCTs on infants and children from 1 month to 5 years of age given vitamin D (in any dose or regimen or route) as an adjunct to standard antibiotic therapy for acute CAP and compared to placebo (or nothing). All aetiologies of pneumonia (except aspiration pneumonia) and all degrees of severity were included. The protocol for this systematic review and meta-analysis has been registered with PROSPERO 2014:CRD42014010259.

We only included trials which had used either of the two diagnostic criteria for acute CAP as given below:

i) Diagnosis of acute CAP by a physician on the basis of clinical examination and/or radiological features; OR,

ii) History of cough or respiratory distress with a respiratory rate above the World Health Organization (WHO) defined age-specific respiratory rate of ≥ 50 breaths per minute for children aged two to 11 months, or respiratory rate ≥ 40 breaths per minute for children aged 12 to 59 months); and either documented fever of >101°F ~~and~~ or chest in-drawing [16].

Trials on patients with congenital abnormalities, rickets, and protein-energy malnutrition or exclusively on proven fungal pneumonia were excluded. We also excluded trials in which vitamin D was administered along with other vitamins or supplements. The review included all papers irrespective of language or publication status.

The primary outcomes we considered were:

**Met opmerkingen [ML5]:** Please add a reference for this statement.

**Met opmerkingen [ML6]:** I would strongly suggest to remove this paragraph on the molecular actions of vitamin D, seems a bit overkill in this meta-analysis which is quite clinical/public health focused.

**Met opmerkingen [ML7]:** Please add reference to the Jolliffe, Griffiths and Martineau systematic review in JSBMB 2013

**Met opmerkingen [ML8]:** Ref. 13 is about nutritional rickets, not necessarily about vitamin D alone. Calcium intake and skeletal effects –rachitic chest development also play a role here. In fact there is not one mention of vitamin D in the results section of ref. 13. Therefore, one could argue that nutritional rickets (which is the most severe form of vitamin D and calcium deficiency and accompanied by pulmonary alterations) plays a significant role in pneumonia risk, whereas less severe vitamin D deficiency or onset of vitamin D deficiency at an older age might not play a role.
Please remove ref. 13 from this statement and discuss this study separately in the context of nutritional rickets (and perhaps explain what nutritional rickets is). Also add the study by Rehman 1994 Journal of Tropical Pediatrics; Salimpour 1975 Arch Dis Child; Najada & Habashneh 2004 Journal of Tropical Pediatrics

**Met opmerkingen [ML9]:** A more balanced account would probably be that most previous meta-analyses have focused on PREVENTION of respiratory tract infections, whereas you are focusing specifically on treatment? Please discuss.

**Met opmerkingen [ML10]:** Please provide the equivalent temperature in °C and mention the 101°F between brackets.

**Met opmerkingen [ML11]:** Why would you exclude that? This is intrinsically linked to vitamin D deficiency and most likely the population where you would expect adjunctive vitamin D to have an effect.
Please also stipulate how many trials were excluded based on this criterion.

**Met opmerkingen [ML12]:** Again, given the background that you provided earlier, this is a population that is also likely very vitamin D deficient. I never expect any effects in populations that are not or only mildly vitamin D deficient. Please specify how many trials were excluded for this criterion and specify the rationale for this criterion.

**Met opmerkingen [ML13]:** Were there any trials combining calcium and vitamin D? That would make a lot of sense. Again, please provide details in the manuscript on how many trials used vitamin D in combination with other supplements.

i) Clinical cure rates which was defined as clinical recovery (that is no fever, no tachypnoea and no chest in drawing) by the end of treatment;
ii) Time- to- clinical recovery of pneumonia;
iii) Time- to- radiological resolution ~~of symptoms~~; and
iv) All-cause mortality.

The secondary outcomes we considered were:
i) Total duration (in hours) of hospital stay (time from randomisation to discharge);
ii) Requirement of any additional interventions like mechanical ventilation, steroids, ~~vaso-pressure~~vasopressor agents, or anything else;
iii) Rates of following complications of pneumonia: parapneumonic effusions and empyema, necrotising pneumonia, and septicaemia and metastatic infection.

We searched for the trials in four electronic databases adopting the following PubMed search strategy "(((((vitamin D) OR Cholecalciferol) OR Ergocalciferol) OR calcitriol)) AND (pneumon* OR bronchopneumon*)" without restricting for language or date:
1. PubMed (last searched 20 June 2016)
2. Cochrane Central Register of Controlled Trials (CENTRAL) (last searched 20 June 2016)
3. CINAHL (last searched 20 June 2016)
4. Global Health (last searched 20 June 2016)

We searched two clinical trial registries (ClinicalTrials.gov and WHO ICTRP) ~~by using the following search strategy~~ using the following search strategy "Pneumonia AND (vitamin D OR cholecalciferol OR ergocalciferol OR calcitriol)" in June 2016. We also searched for grey literature by contacting researchers and searching abstracts of scientific meetings and references of included trials found by other methods.

Two review authors independently screened all the search results initially for consideration of inclusion as per eligibility criteria based on title and abstracts. After the initial screening full texts were obtained and assessment for inclusion and extraction was done by two authors independently. The risk of bias assessment on each study was independently conducted by two authors ~~independently~~ according to the methodology laid down in the Cochrane Handbook for Systematic Reviews of Interventions [17]. Any discrepancies were resolved by consensus.

We used risk ratios (RR) for dichotomous data and mean difference (MD) for continuous data with 95% confidence interval (CI) using Review Manager 5 software [18]. Where data was sufficient, we combined data using intention-to-treat (ITT) analysis and calculated a summary statistic for each outcome by a meta-analysis. The statistical heterogeneity was determined by a combination of visual inspection of graphs of RRs as well as using the $I^2$ statistic [17], and the $Chi^2$ test.

Where appropriate we conducted meta-analyses. We used a used a random-effects model if studies were statistically heterogeneous, otherwise we used a fixed-effect model. When random- -effects model was used, we additionally conducted a sensitivity check using a fixed- -effects model to understand differences in results [17].

We had planned to conduct a sub-group analysis if sufficient number of trials were found for several parameters (severity of pneumonia, dosage, frequency, type and route of antibiotics used and dosage, frequency and route of vitamin D supplementation) but this was not possible. We summarised the main findings of the review in the summary of findings table using the GRADE approach [19].

**Results**

The literature search identified a total of 323 papers (PRISMA Diagram Figure 1), of which four trials met the eligibility criteria, of which two have been completed [20,21] and the remaining two are ongoing (NCT02054182, NCT02185196). The main features of the completed trials from India and Afghanistan are

**Met opmerkingen [ML14]:** End of treatment with vitamin D?

**Met opmerkingen [ML15]:** Radiological findings are never a symptom.

**Met opmerkingen [ML16]:** What is the time frame for this outcome?

**Met opmerkingen [ML17]:** This term is no longer recommended: it is a mash-up of bacteremia and sepsis, which could be examined as separate outcomes. However if the included RCTs mention 'septicemia' rates as an outcome this is acceptable for the meta-analysis.

**Met opmerkingen [ML18]:** From which scientific meetings did you search the abstracts? Please specify.

summarised in Table 1. The ongoing trials are being conducted in South Africa and Bangladesh and the characteristics of these trials as evident from the clinical trial registries ~~is~~ are presented in Table 2.

The completed trials from India [20] and Afghanistan [21] ~~had~~ differed in terms of population (age, severity of pneumonia), as well as dosage and duration of vitamin D supplementation. The study from India [20] included children of 2 months to 5 years of age with severe pneumonia. This classification was bases on the Indian Association of Paediatrics classification of severe pneumonia. These children were supplemented with oral Vitamin D (1000 IU to children less than a year and 2000 IU to children between 1-5 years of age) for five days [21]. The trial from Afghanistan [22] included children of 1 week to 3 years of age presenting~~ed~~ with non-severe, severe and very severe pneumonia (as per the WHO criteria [16]). These children were given a single dose of 10,000 IU of vitamin D orally. The trial from Afghanistan [22] excluded children with ~~R~~rickets whereas~~,~~ five of the included children in the Indian trial had ~~R~~rickets [21]. Baseline and follow-up vitamin D status was not biochemically determined in either RCT.

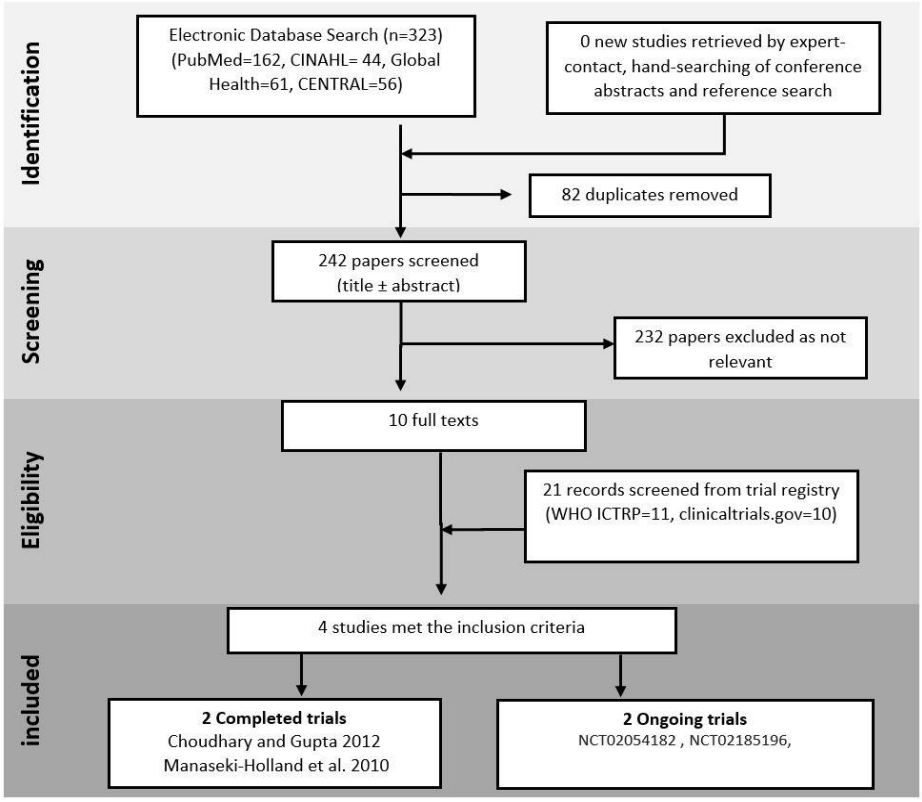


**Figure 1 : PRISMA flowchart showing the inclusion of studies**

**Met opmerkingen [ML19]:** Do you have a reference for this classification?

**Met opmerkingen [ML20]:** In your methods you specified that you would exclude RCTs investigating children with rickets. The trial from Afghanistan also excluded this population whereas this trial did not.
I am curious as to whether you excluded any studies specifically for having many children with rickets and in that case, I would in fact suggest that you include those studies.

**Met opmerkingen [ML21]:** 1.If I count correctly then n=323 minus 82 duplicates gives 241 unique papers, not 242. Which number is correct?
2. Please provide the 10 fulltexts you screened for eligibility as Supplementary data for Editorial review.

**Table 1 : Characteristics of completed trials**

| Study and year of publication | Country and setting | Participants | Intervention | Control | Other Therapy | Outcomes reported and their definitions | Comments |
|---|---|---|---|---|---|---|---|
| **Choudhary and Gupta 2012** | India<br>Tertiary care Hospital | **Age:** 2 months to 5 years<br><br>**Inclusion:** Children with a clinical diagnosis of severe pneumonia, presenting to paediatric emergency department.<br>***Definition of Pneumonia:*** Children with fever, cough, tachypnoea and crepitation were diagnosed with pneumonia.<br>***Definition of Tachypnoea***: Tachypnoea was defined as respiratory rate ≥50/min in children between 2-12 months and ≥40/min in 1-5 years age group.<br>***Definition of Severe Pneumonia:*** Those with pneumonia and either chest in drawing or at least one other danger sign (inability to feed, lethargy, and cyanosis) were diagnosed as having severe pneumonia.<br><br>**Exclusion:** Children with severe wasting (weight for height <3SD), chronic illnesses, previous history of vitamin D intake over last 4 weeks, and known asthmatics were excluded. | Vitamin D (n=100)<br><br>**Dosage:** 1000 IU to children < one year of age and 2000 IU to children between 1-5 years of age.<br>**Delivery:** The drug/placebo was dispensed in milk.<br>**Route:** Oral. Those unable to take orally were given the drug by nasogastric tube.<br>**Frequency:** First dose within four hours of admission. This was followed by once-a-day dosing.<br>**Duration:** 5 days | Placebo (n=100) | Both the groups received antibiotics as per Indian Academy of Paediatrics (IAP) guidelines and supportive care (oxygen, intravenous fluids and monitoring). Children with associated wheezing received salbutamol nebulization twice at an interval of 20 minutes. | • **Time to resolution of severe pneumonia:** Resolution of severe pneumonia was considered when lower chest retraction and the danger signs (inability to feed, lethargy, cyanosis or hypoxia) were no longer present.<br>• **Duration of hospitalization**: The duration of hospitalization was defined as the time (in hrs) between study enrolment and discharge.<br>• **Time to resolution of tachypnoea**: respiratory rate cut off for severe pneumonia as per age.<br>• **Time to resolution of chest retractions.**<br>• **Time to resolution of Hypoxia**<br>• **Time to resolution of Fever:** axillary temperature <37.5ºC<br>• **Time to resolution of inability to feed**: oral feeding had resumed, for a minimum period of 24 hours.<br>• **Discharge from Hospital:** The patient was considered fit for discharge when he/she was afebrile (axillary temperature <37.5ºC), tachypnoea had subsided, there were no chest in drawings, and oral feeding had resumed, for a minimum period of 24 hours. | 5 children had clinical evidence of rickets and they were given a mega dose of vitamin D (6, 00,000 IU) at the time of discharge. (rickets; 2 in vitamin D and 3 in placebo group)<br><br>33.5% of the enrolled children had ~~w~~wheeze.<br><br>30% of the children in intervention and 33% of the children in placebo arm had past history of pneumonia<br><br>**Funding** None |
| **Manaseki-Holland 2010 ISRCTN61245920** | Kabul, Afghanistan Outpatient clinic. | **Age:** 1 week to 3 years of age<br><br>**Inclusion:** Children diagnosed clinically with 'pneumonia' (non- | Vitamin $D_3$ (n=224) | Placebo (n=229) | Children were treated with antibiotic according to the national | • **Recovery:** For two consecutive days, respiratory rate <40 / min, no danger signs or subcostal recession, and no fever. | The study was funded by the |

**Met opmerkingen [ML22]:** Please also specify the mean age from both studies

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | severe or severe) at the local Maywand Teaching Hospital were eligible for inclusion in the trial.<br>***Definition of Pneumonia:*** (i) Age-specific tachypnoea (>60/min if <2 months; >50/min if 2–11 months; >40 if 12– 24 months) and (ii) absence of wheeze (with or without fever).<br>***Definition of Severe pneumonia:*** above-mentioned criteria of pneumonia plus chest in drawing.<br>***Definition of Very severe pneumonia:*** criteria of pneumonia plus at least one of the danger signs (central cyanosis, severe respiratory distress [head nodding, nasal flaring, grunting], inability to drink, convulsions, vomiting).<br>***Definition of Fever:*** Axillary temperature >37.50 °C (age 1 week-3 months) or >38.0 °C (2-23 months).<br>**Exclusion:** Children who had clinical signs of rickets or were known to have received high-dose vitamin D treatment in the past 3 months (one child) had severe vomiting (one child) or pronounced wheeze (10 children) were excluded from the study. (Children who developed wheeze after enrolment were not excluded.). Thirteen children with very severe pneumonias and nine children with other severe illnesses (meningitis, heart or renal disorders, measles, severe malnutrition and suspected tuberculosis) were also Excluded | **Dosage:** 10 000 IU<br>**Delivery:** The drug/placebo was dispensed in olive oil.<br>**Route:** Oral.<br>**Frequency:** Single dose | | pneumonia treatment protocol [based upon Integrated Management of Childhood illnesses (IMCI) guidelines] | • **Failure to treat:** No reduction in the resting respiratory rate over a 72 h period compared to that detected at enrolment after allowing for a variability of ±5 breaths ⁄ min of the baseline respiratory rate<br>• **Repeat episodes of pneumonia:** An episode of pneumonia 14 days after the last day of illness of the previous episode of pneumonia | New Zealand Aid Cooperation. |

**Table 2 : Characteristics of ongoing trials**

| Study and year of publication | Study Design | Country and setting | Participants | Intervention | Control | Other therapy | Outcomes and their definitions |
|---|---|---|---|---|---|---|---|
| NCT02054182 | RCT | Pretoria, South Africa Dr George Mukhari Academic Hospital | **Inclusion:** All children age 1 month – 5 years, admitted in the paediatric unit , with an acute lower respiratory tract infection i.e. bronchiolitis and/or pneumonia<br>**Exclusion:** Children whose caregivers decline participation in the study. Children with co-morbid chronic respiratory condition(s). Children who have received vitamin D supplementation in the past 30 days. | Vitamin D (n=160 )<br>**Dosage:** 2500 IU.<br>**Route:** oral<br>**Frequency:** Daily<br>**Duration:** From enrolment to discharge | Placebo (n=160) | Standard Therapy | **Primary**<br>• Comparison of change from baseline in modified Respiratory Distress Assessment Instrument score at hospital discharge<br>**Secondary**<br>• Comparison of duration of hospitalization: from the day of admission to the day the child is assessed and deemed fit for discharge by the attending physician |
| NCT02185196 | RCT | Bangladesh | **Inclusion:** Children aged 3- 59 months with severe pneumonia(clinically diagnosed) with or without diarrhoea<br>**Exclusion:**<br>• Children who have received vitamin D or calcium supplements within last 4 weeks,<br>• Known to have hypercalcemia or allergy to vitamin D<br>• Congenital heart disease<br>• known case of tuberculosis<br>• renal or hepatic insufficiency<br>• known case of tuberculosis<br>• critically ill children, requiring ICU care, such as those with septic shock or cardia arrest or apnoea<br>• any children diagnosed to have hypernatremia during the main phase of the study | Vitamin D3 (n=175)<br>**Dosage:** 20 000 IU in children <6 month ; 50,000 IU in children 6-12 month ; 1,00,000 IU in children 13-19 months on first day and thereafter 10,000 IU for next 4 days<br>**Route:** Oral<br>**Duration:** Single dose | Placebo-Miglyol-oil (n=175) | standard antibiotic and other supportive therapy | **Primary**<br>• Time to recover from severe pneumonia<br>**Secondary**<br>• Duration of hospitalisation<br>• Time taken for normalisation of temperature<br>• time taken for normalisation of respiratory rate<br>• Time taken for recovery from chest in drawing<br>• Time taken for oxygen saturation to normalise<br>• Time taken for normalisation of child feeding<br>• Proportion of children who will develop new episode of pneumonia during 12 months of follow-up |

The trials were judged to be low risk of bias for most parameters except some. Figure 2 and Figure 3 provides a graphical summary of the risk of bias and the basis for these assessments is provided in Table 3.

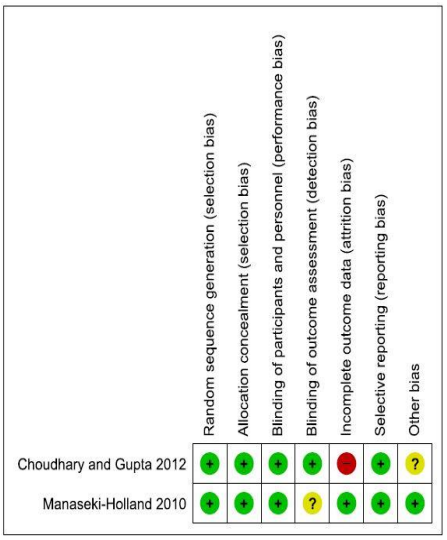


**Figure 2 Risk of bias summary: review authors' judgements about each risk of bias item for each included study.**

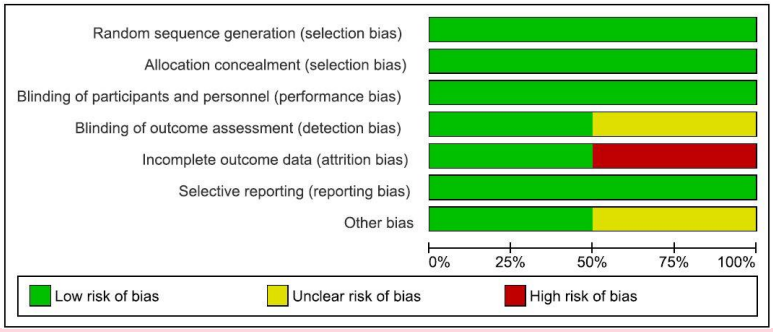


**Figure 3 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.**

**Met opmerkingen [ML23]:** I suggest removing this, since you have only 2 trials I think this isn't common sense.

**Table 3 : Rationale for risk of bias of included trials**

| ROB DOMAIN | Choudhary and Gupta 2012 | Manaseki-Holland 2010 |
|---|---|---|
| **Random sequence generation (selection bias)** | **Low risk**<br>"Computer generated random number table." Authors were not involved with the randomization process | **Low risk**<br>"random number sequence generated in an Excel spreadsheet with no restrictions" |
| **Allocation concealment (selection bias)** | **Low risk**<br>"Allocation concealment was done by sealed envelope Technique" | **Low risk**<br>"….blinded doctors choosing the next syringe with a randomisation code." |
| **Blinding of participants and** | **Low risk** | **Low risk** |

| | | |
|---|---|---|
| **personnel (performance bias)** | "double blind" ; "Both the caretaker and subject were blind regarding the content of the drug been given" | "Double blind"; "Placebo (Containing olive oil alone) and vitamin D syringes looked the same and the contents tasted the same. None of the investigators, staff in Kabul and caretakers of children were aware of the study groups." |
| **Blinding of outcome assessment (detection bias)** | **Low risk** "Both the caretaker and subject were blind regarding the content of the drug been given"; "The code key was opened only after the intervention, data collection; follow up and tabulation were completed." | **Unclear risk** Insufficient information to permit judgement. Trial author could not be contacted for further information. |
| **Incomplete outcome data (attrition bias)** | **High risk** "A total of 7 children could not complete the study as parents left against medical advice (Figure 1). There was no difference between the two groups in the proportion of children who improved." Comment: Figure 1 in the study does not detail this information. No further information provided on contact with trial author | **Low risk** 204/224 = 8.9% in Vitamin D group and 211/229 = 7.9% from placebo group were loss to follow up and reasons were almost similar. |
| **Selective reporting (reporting bias)** | **Low Risk** All outcomes are reported as mentioned in the methods section. No trial registration was done, | **Low risk** Outcomes are reported as mentioned in the objective and trial registry. |
| **Other bias** | **Unclear Risk** Trial was not registered and no protocol published. Funding : None | **Low risk** Trial is registered. ISRCTN61245920 Funding: New Zealand Aid Cooperation |

**Effect of intervention**

**Clinical cure rates:** Only the trial from India has reported clinical cure rates in children and they found no significant difference in clinical cure rates (RR 1.01; 95% CI 0.91, 1.13). (Figure 4) The trial conducted in Afghanistan did not report on cure rates.

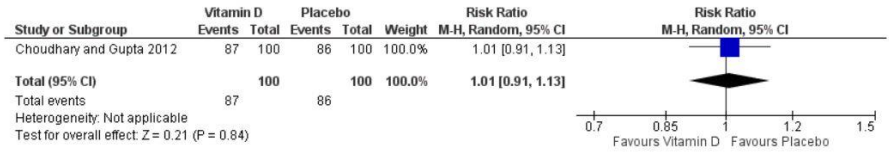


**Figure 4 : Forest plot for Clinical Cure Rates: Vitamin D versus Placebo**

**Time-to-clinical recovery of pneumonia:** Both the trials reported this outcome but they could not be pooled due to differences in reporting. In the trial from India, the median time of recovery in the vitamin D group was 72 hours (standard error (SE), 95% CI 48-96 hours) and 64 hours (SE, 95% CI 48-88 hours) in the placebo group (P 0.33). The time to clinical recovery in the trial from Afghanistan was similar across vitamin D and placebo groups (4.74 days ± 2.22 in vitamin D vs. 4.98 days ± 2.89 in placebo; P 0.17) (Table 5). We could not carry pooled analyses since data to do analyses of this time-to-event outcome was not available.

**All-cause mortality:** Both the trials reported all-cause mortality, but these trials were heavily underpowered for this outcome with only 3 events in the vitamin D and 2 events in the placebo group in both trials together. Overall, ~~and~~ children with vitamin D supplementation has~~d~~ a non-significant

1.5 ~~times the~~ risk of death ratio compared to children with no supplementation (RR 1.52; 95% CI 0.26, 9.02). A fixed effects model was used since no heterogeneity was detected. (Figure 5) (Table 5).

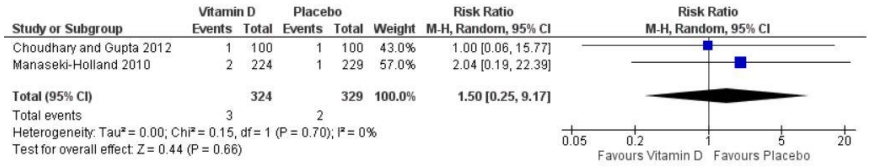


**Figure 5 Forest plot for all cause-mortality: Vitamin D versus Placebo**

**Total duration of hospital stay:** Only the trial from India reported this outcome. The median duration of hospitalization was 112 hours (interquartile range (IQR) 96-136) in vitamin D group versus 104 hours (IQR 88-128) in placebo group (P 0.29) [20] (Table 5).

None of the included study reported any data on **requirement of any additional interventions and complications of pneumonia**. Additional data on these outcomes were not provided by authors of the Indian trial [20] and authors from the Afghanistan trial could not be contacted [21].

None of the trials reported any data on the outcomes of time to radiological resolution ~~of symptoms~~, requirement of any additional interventions and rates of complications. No trials reported any safety outcomes e.g. incidence of hypercalcemia.

We had intended to carry on sensitivity analysis and conduct a funnel plot for estimating the publication bias, however due to the limited availability of trials this was not possible. The GRADE summary of findings table for the primary outcomes is shown in Figure 6. We found low quality of evidence for the outcomes of clinical cure rates and all-cause mortality for both the outcomes.

**Met opmerkingen [ML24]:** Please specify:
Population: infants and children up to 5 years of age
Settings: hospital settings were only analysed in the systematic review

**Vitamin D Versus Placebo for Community Acquired Pneumonia in infants and children**

**Patient or population:** patients with Community Acquired Pneumonia in infants and children
**Settings:**
**Intervention:** Vitamin D Versus Placebo

| Outcomes | Illustrative comparative risks* (95% CI) Assumed risk Control | Corresponding risk Vitamin D Versus Placebo | Relative effect (95% CI) | No of Participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| Clinical cure rates | Study population | | RR 1.01 (0.91 to 1.13) | 200 (1 study) | ⊕⊕⊖⊖ low[1,2] | |
| | 860 per 1000 | 869 per 1000 (783 to 972) | | | | |
| | Moderate | | | | | |
| | 860 per 1000 | 869 per 1000 (783 to 972) | | | | |
| All cause mortality | Study population | | RR 1.5 (0.25 to 9.17) | 653 (2 studies) | ⊕⊕⊖⊖ low[3] | |
| | 6 per 1000 | 9 per 1000 (2 to 56) | | | | |
| | Moderate | | | | | |
| | 7 per 1000 | 10 per 1000 (2 to 64) | | | | |

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

**CI:** Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
**High quality:** Further research is very unlikely to change our confidence in the estimate of effect.
**Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
**Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low quality:** We are very uncertain about the estimate.

[1] Down graded for serious risk of bias because of high risk of attrition bias in study.
[2] Downgraded for serious imprecision because total number of events is less than 300
[3] Downgraded for serious imprecision since there is wide confidence interval from 0.25 to 9.17 .

**Figure 6 : GRADE Summary of Findings Table**

**DISCUSSION**

The two completed trials were insufficient to permit judgement about the efficacy and safety of using vitamin D as an adjunct for CAP in infants and children. Differences in clinical cure rates were reported

in the Indian trial [21] only and this was not significantly different between the vitamin D and placebo group. The quality of evidence for this outcome was low. The Indian trial [21] found no differences in duration of hospital stay between vitamin D and placebo group. The RR for mortality on pooled analysis was 1.5 [95% CI 0.25 to 9.17] but is of low quality. The improvement in time - to - clinical recovery was also not significant. However, none of the included trials reported any data on requirement of any additional interventions and other rare complications of pneumonia and hence the safety of the therapy could not be commented upon.

This systematic review has used explicit, pre-specified methods in the process and we have searched for RCTs in multiple electronic databases and by other methods. We believe that no relevant trials have been missed. Assessment of publication bias, through funnel plots could not be done because of the small number of studies found.

A recent Cochrane Review [22] on preventive use of vitamin D for infectious diseases in children under five found no evidence to support the use of vitamin D for prevention of pneumonia. Our results, though on different outcomes provides a similar perspective in terms of finding no benefit on using vitamin D as an adjunct during treatment of pneumonia.

**Implications for practice and research**

Current available evidence does not justify the use of vitamin D as an adjunct therapy to antibiotics and standard respiratory support in CAP among infants and children. However, data from observational studies have indicated that vitamin D insufficiency is related to disease severity and prognosis [15, 23, 24]. There is a need to conduct more placebo-controlled double blinded trials with adequate sample size for evaluating the safety and efficacy of vitamin D as an adjunct to antibiotics for childhood pneumonia of varying severity. Since non-severe pneumonia is often managed in communities, community/primary-care based trials should also be conducted. Though identification of aetiological agents is not imperative for the clinical management of pneumonia, – trials should try to identify ~~aetiological agents~~causative respiratory pathogens to evaluate the effect of vitamin D on pneumonia caused by different ~~aetiological agents to be able to provide clues if efficacy might vary depending on aetiology~~microbial organisms.

There are immense differences in the trials (including the undergoing trials- Table 1 and 2) ~~on~~ in the dose ~~for~~ of vitamin D being used~~. Trials have evaluated various dosages of vitamin D –~~, ranging from 1000 IU to 100,000 IU in different regimens and the ongoing trial from Bangladesh has even an age-dependent first dose. The Indian trial used "vitamin D" but did not specify whether or not this was cholecalciferol or not. There is a need to understand the dosage at which vitamin D shows its immunomodulatory functions with respect to pneumonia in infants and children. Future studies should also consider evaluating the underlying vitamin D deficiency status of the populations and quantify the amount of improvement in 25-hydroxyvitamin D level as a result of the ~~use of vitamin D as an adjunct~~intervention.

Vitamin D receptor (VDR) polymorphisms have been shown to affect treatment of tuberculosis in a trial evaluating high dose vitamin D as an adjunct to TB treatment~~. The influence of host gene polymorphism on treatment outcomes was seen in a study~~ [25]~~ it led to faster sputum culture conversion in patients with tt genotype of the Taq1 genotype of the vitamin D receptor polymorphisms~~. Future trials on vitamin D as an adjunct to pneumonia treatment should also investigate the effect of hos~~e~~t gene polymorphisms related to the vitamin D mediated immune response [9] particularly the vitamin D receptor and vitamin D binding protein and CY27B1 polymorphisms.

**Conclusion**

**Met opmerkingen [ML25]:** Please expand here. One of the trials you discuss, Manaseki-Holland 2010, did find lower risk and longer time to repeat pneumonia. However when pooled this apparently did not hold up. It would be useful to recapitulate some of the findings from the studies in the closely related Cochrane report to give the reader an idea about what vitamin D might be good for in prevention of pneumonia.

**Met opmerkingen [ML26]:** Please read this paper:
Grant WB, Mascitelli L, Goldstein MR. Proposed Guidelines for Future Vitamin D Studies. JAMA Intern Med. 2016;176(2):279-80. And discuss it in the section i.e. speculate on possible reasons for the lack of effect of vitamin D in these studies.

**Met opmerkingen [ML27]:** Based on the results of these two trials, can you calculate what the sample size should be for different (large, medium, small) expected effect sizes? I.e. using the event rates and standard deviations observed.

**Met opmerkingen [ML28]:** Please expand here on the fact that there is some evidence for a role of vitamin D in resistance to tuberculosis whereas prevention trials for viral infections appear negative.

**Met opmerkingen [ML29]:** Please add discussion from the intensive care literature in adults that large doses may be required to efficiently correct vitamin D deficiency in the acute setting.

Secondly, given that you suddenly introduce vitamin D-binding protein in the next paragraph, it would be useful to underline the importance of vitamin D-binding protein in the assessment of vitamin D status particularly in the African population, and also its confounding role in acute infectious situations such as seen in sepsis.

Thirdly, a limitation of the use of vitamin D for adjunctive treatment is the relatively short time span in which you expect the effect. Therefore prevention trials, in which there is time to correct vitamin D BEFORE the pneumonia occurs, might be more useful. Along the same lines, it would be interesting to have trials SPECIFICALLY in the rickety population with pneumonia as an outcome (although placebo use would be difficulty to justify in this population). Please add discussion of these elements.

Vitamin D is cheap and if proved safe and effective as an adjunct to pneumonia therapy, it would provide~~s~~ an immense opportunity from the point of view of public health to tame the high burden of the childhood mortality and morbidity due to CAP. Similar immunological mechanisms are in play in various other infectious diseases [8, 9, 26] and their role in diarrhoea [27] and malaria [28] is being investigated~~ion~~ from the point of view of childhood mortality and morbidity. There is an immense need for more RCTs on this issue in diverse settings taking into care aspects of clinical trial design highlighted vide this systematic review.

**Conflict of Interest:**
None

~~**Acknowledgements:**~~
~~None~~

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**Met opmerkingen [ML30]:** If you have no acknowledgements this can be removed.

**Met opmerkingen [ML31]:** There are multiple different styles for reference formatting here. Please provide PubMed ID numbers (PMID) for all references, this will facilitate later processing for publication.

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