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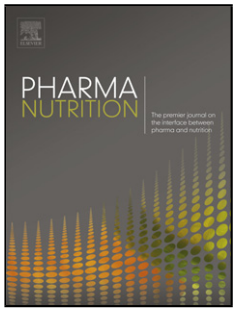

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The effect of vitamin D supplementation on inflammation in critically ill patients: a systematic review

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Abstract

Purpose: Vitamin D intervention may affect the immune system function and modulate the innate and adaptive responses in relation to the status of patients with critical illness.

Methods: The search terms were conducted on PubMed, Cochrane Library, EMBASE, Scopus, clinical trials and gray literature databases and clinical trial studies published from 2000 to July 2019 were included in the present study. Two independent researchers selected 53 studies that examined vitamin D supplementation in critically ill patients. Three researchers assessed study designs, subjects, interventions, outcomes, and data quality according to the Cochrane scoring system.

Results: Three randomized clinical trials of critically ill patients treated with high dose of vitamin D supplements indicated consistent reductions in pro-inflammatory cytokines (interleukins 1 and 6). Five clinical trials illustrated no significant differences in C-reactive protein levels between vitamin D and placebo groups. Outcomes of secondary analyses in two trials showed no significant reduction in interleukin 6 levels (pooled effect size, IL-6, -16.32 [-40.78, 8.15]) while treated with high dose of vitamin

D supplements. Moreover, vitamin D supplementation indicated no considerable effects on CRP levels in recipient group versus non-recipient group (pooled effect size -2.65 [-18.02, 12.72]).

Conclusions: Evidence from few clinical studies suggests that high doses of vitamin D interventions may reduce pro-inflammatory cytokines, whereas it appears to have no significant effects on anti-inflammatory cytokines and C-reactive protein levels. Further well-designed research studies are required to elucidate the effect of vitamin D supplementation on immune responses in critically ill patients.

Keywords: Vitamin D, Cholecalciferol, Cytokines, Inflammation, Critically ill

1. Abbreviation list:

AM: Arabi, Mostafa

BL: Bahrami, Leila

NA: Norouzy, Abdolreza

GR: Ranjbar, Golnaz

IL: Interleukins

CRP: C-reactive protein

Hs-CRP: High sensiviety C-reactive protein

INF: Interferon

TNF: Tumor necrosis factor

RCT: Randomized clinical trials

PICOS: participants, Intervention, Comparison, Outcome, Study type

2. Introduction

Vitamin D is a fat-soluble vitamin that is produced by the body in primary form when exposed to sunlight in the skin (pre vitamin D3) and is then converted to 25 (OH) vitamin D3 in the liver and 1,25 (OH) vitamin

D3 in the kidneys, respectively (1, 2). Low levels of circulating active vitamin D could cause many medical complications, since it has various functions; including calcium and bone metabolism, immune responses, and inflammatory processes (3, 4). Additionally, previous interventional studies have shown that supplementation with vitamin D can reduce the levels of inflammatory markers and regulate the balance of inflammatory-anti-inflammatory parameters (5). Although there is a need for more extensive studies in this field, several studies have suggested that vitamin D may possess beneficial effects on reducing the risk or adverse consequences of metabolic stress, including critically ill, autoimmune and inflammatory diseases (6, 7). Vitamin D is associated with a large number of diseases in general population (8). According to several observational studies, vitamin D deficiency is prevalent among critically ill patients, which itself can be a potential factor for increasing the risk of infection, inflammation, pneumonia and mortality rate (9, 10). It has also been observed that vitamin D levels were reduced over the time of hospitalization (11). Pascal et al., conducted a meta-analysis and systematic review study, they showed no effect of vitamin D supplementation in clinical outcomes of critically ill patients (12). Whilst, a number of studies have been conducted in this regard; the role of vitamin D in critically ill patients is still unclear. Thus, this systematic review aimed to summarize the current RCTs conducted on vitamin D supplementation and critically ill patients in order to evaluate the effect of vitamin D supplementation on inflammatory markers in these patients at ICU.

3. Methods

2.1 Search strategy and study identification:

A systematic search was carried out in accordance with the PRISMA Checklist (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The search terms were carried out by two independent researchers (AM, LB) in PubMed, Cochrane Library, Embase, Scopus, clinical trials and gray literature databases. All of the articles were published from December 2000 to July 2019. The search terms were not limited by the language of publication, the key words and MeSH terms were also selected as follows: [redacted].

[redacted]

[redacted]

reference lists of the relevant articles were also reviewed to ensure adequate study identification.

Thereafter, the titles and abstracts of the most relevant articles were screened and duplicate publications were deleted. Finally, all the relevant articles in full-text were obtained and read thoroughly.

2.2 Included Studies

A structured approach was taken to set up the research question about this review, using the following five components that are commonly known as the Participants, Interventions, Comparisons, Outcomes, and Study Design Approach (PICOS) (14):

[illegible] old who were admitted to the intensive care unit (ICU), surgical and non-surgical ICU patients. There was no restriction on their sex, age, race and geographical distribution of the participants. Table 1 shows the inclusion and exclusion criteria.

2. Interventions: Enteral or intravenous, intramuscular vitamin D administration in the form of active vitamin D (calcitriol) or vitamin D3 (cholecalciferol).

3. Comparisons: Placebo or low dose of vitamin D supplementation.

4. Outcomes: All of the trials must include main outcomes, including inflammatory cytokines (interleukins, interferon gamma and tumor necrosis factor alpha) and inflammatory markers (C-reactive protein, Fibrinogen and procalcitonin) (table 1).

5. Study design: Randomized controlled trials (RCTs).

2.3 Excluded Studies:

1. Observational studies, review articles, editorials, case reports, letters to the editor and animal studies.

2. Studies that included vitamin D with other supplements or combination therapy with specific drugs.

2.4 Eligibility review and data abstraction

Two independent researchers fulfilled the data extraction and they were blinded from the other author's report during the process (AM and LB). The quality and quantity of information abstraction was elicited

from each study. Disagreements on the inclusion of a study between the reviewers were resolved by adjudication of a third reviewer (AN). The P - value of <0.05 was considered statistically significant for all of the studies. Data extracted from each study was summarized according to the name of the first author, year of publication, country of origin, study design, sample size, dose of vitamin D and types of vitamin D supplements, main outcome and the conclusion. P-value at significance level of 0.05 for inflammatory markers between intervention and placebo groups were included for meta-analysis. In order to receive the statistical information that were not reported in the selected articles, we contacted their corresponding authors through emails.

2.5 Risk for Bias and publication bias Assessment

We evaluated each trial for quality and risk of bias using the Cochrane scoring system (15). The following variables were used to determine the risk of bias: sequence generation, allocation concealment, blinding, blinding of outcome assessment, incomplete outcome data and selective reporting. Each item was labeled as (+) with low risk of bias, (x) with high risk of bias and (?) with unclear information. We also assessed the

Egger et al. Also, the fil

potential effects of publication bias (16).

2.6 Statistical analysis

All analyses were performed using RevMan 5.3 software (Cochrane IMS, Oxford, UK) with a random effect model (17), except the calculation of publication bias that was assessed by Comprehensive Meta-Analysis Software (CMA) version 2. The DerSimonian and Laird random effects model were used to estimate the Mantele-Haenszel and the inverse variance estimators' variances (18).The mean and standard deviation between the effect of experimental vitamin D supplementation and control groups on inflammatory markers were used as the effect size for meta-analysis. According to Cochrane method, summary weighted

statistic (I^2) (18, 19).When intervention existed more than twice, and we analyzed each event separately. In a study conducted by Amerin et al.,(6) CRP was assessed in day 3 and 7, in addition, in a study by Leaf et al, ,(20) IL-6 was evaluated at 6 hours after treatment and in day 1 and 2.

4. Results

In this systematic review, 2200 articles were found after initial screening, only 53 full-text articles were included for revision and 48 of these studies were excluded as they were In vitro studies and lacked clinical trial design, human subjects and ICU patients. Therefore, five RCT studies were included in this systematic review, a total of 645 critically ill adults aged 18 70 years old were included in this pooled analysis. The reviewers achieved 100% consent to the inclusion of these studies. PRISMA flow chart-diagram for the study selection process is illustrated in figure 1 (13). The main characteristics and outcomes of the five RCTs included in the present study are presented in table 2 (6, 20-23). In summary, the experimental duration in these studies ranged between 48 hours and 2 weeks.

3.1.1 Study Characteristics

Two RCTs were carried out in the United States (20, 23), two in Australia (6, 22), and one in Iran (21). The effect of vitamin D3 supplements on CRP and IL-6 were evaluated in four of these studies (6, 21-23). Other markers including IL-2, IL-4, IL-10, interferon gamma, procalcitonin and fibrinogen levels were also assessed. However, since there was an insufficient numerical data, a descriptive analysis was carried out in this regard. For instance, in Quraishi et al., IL-s> , IL-6, TNF-=
(23).

3.1. 2 Participant Characteristics

A total of 645 critically ill adult patients were admitted to the intensive care unit (ICU). In the first 24 to 48 hours of admission, patients were reported to have a wide range of clinical diagnosis, including cardiovascular and cardio surgical (29%), sepsis (23%), neurological (17%), trauma (8.5%), other conditions (22.5). Males consisted 62%

3.1. 3 Intervention Characteristics

Different doses of vitamin D were prescribed in each of these RCTs (one study with 2 mcg intravenous calcitriol) (20), two studies with oral ultra-dose cholecalciferol supplements (6, 23), and two RCTs with 300,000 IU intramuscular injection cholecalciferol (21, 22). Of these five studies, one of them had two intervention groups (400,000 and 200,000 IU) and one placebo group (23).

3.2 Primary analysis:

3.2.1 Descriptive Analyses

Vitamin D Supplementation and IL-1 levels in Randomized Controlled Trials

The results obtained by Leaf et al. (2014) indicated that vitamin D had no significant effects on IL-1 levels versus placebo after 48 hours of supplementation ($p > 0.05$); however, in the study conducted by Quraishi et al. (2015) a significant difference on IL-1 levels was observed between intervention and placebo groups ($p = 0.02$) (table 2).

3.2.2 Vitamin D Supplementation and IL-2 levels in Randomized Controlled Trials

Leaf et al. (2014) also demonstrated that vitamin D supplementation did not change IL-2 levels between the intervention and placebo groups ($p > 0.05$) (table 2).

3.2.3 Vitamin D Supplementation and IL-4 levels in Randomized Controlled Trials

Quraishi et al. (2015), conducted a study on the effect of vitamin D supplementation in sepsis patients, which indicated that differences in IL-4 levels between the intervention group and the control group was not significant at day 1 and 5 of treatment ($p = 0.57$) (table 2).

3.2.4 Vitamin D Supplementation and IL-6 levels in Randomized Controlled Trials

In the study carried out by Quraishi et al. (2015), IL-6 levels decreased significantly in the experimental group between baseline and day 5 of supplementation ($p = 0.02$) (table 2).

3.2.5 Vitamin D Supplementation and IL-10 levels in Randomized Controlled Trials

Vitamin D treatment in critically ill patients versus control patients did not make a significant change on IL-10 levels according to the study conducted by Leaf et al. ($p > 0.05$) (table 2).

3.2.6 [illegible] Controlled Trials

Quraishi et al. (2015) demonstrated that intramuscular cholecalciferol did not significantly change the [illegible] ontrol groups after the intervention ($p = 0.61$). In addition, Leaf et al, illustrated the same results after calcitriol supplementation ($p > 0.05$) (table 2).

3.2.7 [illegible] Controlled Trials

The result of Quraishi study indicated that vitamin D3 intervention caused a similar effect on INF-@ treatment group compared to the control group ($p = 0.09$) (table 2).

3.2.8 Vitamin D Supplementation and hs-CRP levels in Randomized Controlled Trials

According to a study (RCT) by Quraishi et al., vitamin D administration showed no improvement in the levels of hs-CRP in intervention group versus placebo group ($p = 0.59$). Although, the mean change of hs-CRP at day 14 was statistically significant ($p < 0.001$) (table 2).

3.3 Secondary analysis:

Meta-analysis of Randomized Trials

When we merged the data from randomized clinical trials with different duration time of interventions, the pooled effect size for CRP was -2.65 [-18.02, 12.72] with I^2=40% for vitamin D supplements versus control group and the cumulative effect size for IL-6 was -16.32 [-40.78, 8.15] with I^2=88 % for treatment versus placebo group (Figure 2-3).

3.4 Quality and Risk of Bias Assessment

According to Cochrane guideline, the method used for selection bias was appropriate for all of the studies (6, 20-23). Also, performance bias was at low risk in 40% of the studies (21). Whereas, the detection bias was at high risk in 90% of the studies (20-23). The attrition bias and reporting bias of the data in 40% and 90% of the studies received adequate quality, respectively. Studies were regarded as good quality with at least three low risk of bias; studies with two low risk of bias were considered as fair; and studies without or lower than one risk of bias were considered as poor (table 3).

3.5 Publication bias

-6 levels, demonstrated that publication bias was on CRP levels

using ' trim and fill' correction, two potentially missing studies were imputed to left of funnel plots. Therefore, the effect size differs significantly from the initial estimate (adjusted value: 0.13, 95 percent CI -0.21 to 0.48). The' fail- safe N ' test indicated that it would require 21 studies to bring the effect size to non-significant ($P > 0.05$)(16).

5. Discussion

According to the present study findings, the effects of vitamin D supplementation on the inflammatory markers were evaluated in few studies with consistent heterogeneity in vitamin D dose, duration of intervention and study designs. Randomized clinical trials of inflammatory diseases, especially

inflammatory bowel disease (IBD), showed considerable reductions in inflammatory cytokines among those who were treated by vitamin D supplements. However, pervious trials indicated a slight reduction in inflammatory cytokines of critically ill patients treated with high dose of vitamin D supplements (24-26). An active form of vitamin D is directly and indirectly involved in many pro-inflammatory and anti-inflammatory cascades related to the final outcomes of critically ill patients (27). Based on the current data, calcitriol is suggested to modulate cytokine production, and decrease infection and mortality rate (5,26,28,29).

Observational studies have demonstrated an inverse association between low serum calcidiol levels and higher rates of mortality and adverse clinical consequences in critically ill patients (30-32). High doses of vitamin D administration could promote lymphocyte T and dendritic cell differentiation, inhibit cell proliferation and the genes encoding pro-inflammatory cytokines (e.g., IL-1 and TNF) (33-35). Furthermore, it is suggested that vitamin D stimulates T helper 2 response with increase in production of IL-5 and IL-10 levels and reduction in the synthesis of pro-inflammatory cytokines (26, 36, 37). Optimal vitamin D levels could control immune responses, down regulate antigen presentation, suppress production of cytotoxic proteins, inhibit interleukins 1 and 6 synthesis and improve interleukin 10 production from T regulatory cells (38), and finally enhance immune responses to infection in epithelial cells (26). Findings on vitamin D interventions and their effects on inflammatory processes in clinical trial studies have been inconsistent. One study (23) has demonstrated reduction in IL-1 levels and three trials (21-23) showed that IL-6 levels decreased after treatment with high dose of vitamin D supplements. The suppressive effect of vitamin D on the function of pro-inflammatory factors among sepsis and pneumonia patients (25 29) suggests a potential anti-infective function of calcitriol (21-23).

To date, five clinical trial studies have demonstrated the immunomodulatory effect of vitamin D supplements with different doses from 2 mcg to 540000 IU/d in patients with critical illness at ICU (6, 20-23). Two RCTs performed oral vitamin D supplements versus control on critically ill patients, one of them demonstrated a significant reduction in cytokines levels, while the other one did not illustrate any changes in cytokines levels of critically ill patients treated with single dose of 400000 and 540000 IU vitamin D supplements (6, 23). The other two RCT studies tested 300000 IU intramuscular vitamin D versus placebo, and only one of these RCTs indicated a significant reduction in IL-6 levels among groups (21, 22). Moreover, in another study, low dose of intravascular calcitriol versus placebo showed a statistically non-significant

effect on pro- and anti-inflammatory cytokines (20). All narrative and systematic reviews of vitamin D supplementation in critically ill patients to date, have not investigated the effect of vitamin D on inflammation (12, 27, 39). Notably, high doses of vitamin D supplements, whether edible or injected, used in these RCTs have shown an increase in mean plasma calcidiol levels more than 30 ng/ml (6, 20-23). Extrapolating from pervious RCT studies, treatment with vitamin D supplementation in critically ill patients with 25- hydroxyl vitamin D levels at 17.5 ng/ml, caused reduction in the levels of IL-1 and IL-6 in these patients (23). Therefore, further interventions on vitamin D with at least 200000 IU is required to determine whether improvements in proinflammatory cytokines may prevent negative clinical outcomes in critically ill patients. These findings suggest that an anti-inflammatory effect of vitamin D supplementation on inflammation is possible. Despite favorable outcomes of vitamin D supplementation on IL-6 levels from descriptive analyses (21-23) in this systematic review, the findings from the present meta-analysis was inconsistent. According to findings from a clinical trial on severe sepsis patients (10, 14, 30, 31), the active vitamin D form supplements (calcitriol) were unlikely to confer a considerable effect on immunomodulatory markers (20). Also, results from five randomized trials (6, 20-23) have not shown a clear effect of all forms of vitamin D supplementations on plasma C-reactive protein concentration and this finding was consistent with the pooled results from the present meta-analysis on CRP levels. For further justification, firstly, the RCT studies were not exactly designed to evaluate the effect of vitamin D intervention on CRP markers. Secondly, due to the low sample size of the studies (21-23) and their low statistical power, the desired effect may not be statistically significant. However, these studies demonstrated high heterogeneity in the type of disease and supplements form. A recent systematic and meta-analysis study by Mazidi et al., (40) indicated a positive effect of vitamin D supplements on CRP levels between the case and control groups. However, based on present study the positive effects of vitamin D supplementation on CRP levels is not supported in critically ill patients at ICU. The present systematic review has several limitations as follows: few low power eligible studies were conducted on the immunoregulatory effects of vitamin D intervention in critical illnesses. The publication bias must be conducted for interpretation of findings based on published evidence on critically ill patients. However, the [redacted] -analysis results. Finally, participants included in these studies were only ICU patients with different pathophysiology compared to the rest of the patients with other medical conditions, thus the effect of vitamin D supplementation could vary among different patients.

6. Conclusion

In conclusion, few randomized controlled clinical trials have investigated the effect of vitamin D supplementation on inflammation in critically ill patients. To date, published evidence from these studies propose that high doses of vitamin D supplements may have favorable effects on reducing pro-inflammatory cytokines (IL-1 and IL-6) levels, whereas it seems to have no apparent impact on the C-reactive protein status. Further randomized clinical trials that are well-designed with long-term follow-up and large sample size should be conducted to elucidate the potential effects of vitamin D supplementation on pro-inflammatory and anti-inflammatory mediators in critically ill patients.

Declarations section:

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of supporting data

Please contact author for data requests

Competing interests

The authors declare that they have no competing interests.

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Arabi M and Bahrami LS conducted search terms on databases. Norouzy A and Ranjbar, G assessed the quality of studies. Tabesh H analyzed the data. Arabi M designed the manuscript. Bahrami LS revised and Ranjbar, G edited the manuscript.

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Author Contributions Statement

The authors contribution statements were as follows AN designed the study; MA: wrote the first draft of the manuscript; MA and LB: identified and extracted relevant articles and analyzed the data; GR and HT: revised and proofread the manuscript; and all of the authors read and approved the final manuscript. The authors declare no conflicts of interest.

Conflict of Interest: The author(s) declare no competing interests.

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All authors were fully responsible for the validity and reliability of the data, the analysis and the writing of the manuscript.

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Table 1: Inclusion and exclusion criteria of studies

First author, year (ref)	Inclusion criteria	Exclusion criteria	Quality markers(41)
Amerin, 2014(6)	x Critically ill Patients above than 18 years old x Expected to stay in the ICU for 48 hours x Vitamin D levels of 20 ng/mL or lower	x Severely impaired gastrointestinal function x Pregnant or lactating women x Hypercalcemia x Tuberculosis; sarcoidosis; nephrolithiasis within the prior year	C. Random: yes Blinding: double blind ITT: yes
Leaf, 2014(20)	x Critically ill Patients age greater than or equal to 18 years x Severe sepsis or septic shock x Presence of an arterial or central venous catheter	x Hypercalcemia x Hypophosphatemia x Parathyroid disease x Metabolic bone disease, sarcoidosis, or end-stage renal disease x AKI receiving intermittent or continuous RRT x Pregnancy	C. Random: yes Blinding: double blind ITT: yes

Quraishi, 2015 (23)	x Critically ill Patients at least 18 years old x Sepsis or septic shock	x Current vitamin D supplementation x Anemia at the time of ICU admission x Hypercalcemia x Pregnant or immediately postpartum women x High gastrointestinal output x High likelihood of dying within the first 48 hours of ICU admission	C. Random: yes Blinding: no ITT: yes
Nair, 2015 (22)	x Adult critically ill Patients x Systemic inflammatory response syndrome	x Severely impaired gastrointestinal function x Pregnant women x Hypercalcemia x Sarcoidosis; lymphoma, or multiple myeloma and chronic kidney disease x Coagulopathy	C. Random: yes Blinding: single blind ITT: yes
Miroliaee, 2017(21)	x Adult critically ill Patients x Pneumonia after mechanical ventilation	x Chronic renal failure x Pancreatitis x Hepatic insufficiency x Coagulopathy	C. Random: yes Blinding: double blind ITT: no

- x Cancer or chemotherapy

Legend: intensive care unit (ICU); intention to treat (ITT); computerized (C)

Table 2: Randomized controlled studies comparing the effect of vitamin D supplementation versus placebo in criticality ill patients

Study	Population	Duration of study	Vitamin D dose and type in intervention group	Comparator group treatment	Biomarkers	Outcome	Conclusion
Amerin. 2014,Australia	492 critically ill subjects (F=166 M=309).	1 week	540 000 IU oral solution of vitamin D3	Same volume oleum arachidic from olive oil	CRP	CRP ↔	No effect
Leaf. 2014, USA	67 critically ill subjects with sepsis (F=30 M=36).	48 hours	2 mcg Intravenous calcitriol	2 ml Intravenous saline	IL-1B, IL-6, IL-10, TNF	IL-1 ↔ IL-2 ↔ IL-6 ↔ IL-10 ↔ TNF ↔	No effect
Nair.2015, Australia	x 50 critically ill subjects with hypn	2 week	200 000 IU Intramuscular vitamin D3	150 000 IU Intramuscular vitamin D3	CRP, IL-6	CRP ↔ IL-6 ↓	No effect

	eu mo nia (F= 14, M= 36) .						
Quraishi.2015,USA	20 critically ill subjects with sepsis (F=8 M=12).	1 week	400 000 IU and 200,000 IU oral solution of vitamin D3	Placebo liquid	hs-CRP, IL-1B, IL-4, IL 6, TNF- r U / E &	hs-CRP ↔ IL-6 ↓ IL- í t ↓ IL-4 ↔ TN[illegible] ↔ I[illegible] v ↔	No effect on inflammation
Miroliaee.2017, Iran	46 critically ill subjects with pneumonia (F=17 M=29).	1 week	300 000 IU Intramuscular vitamin D3	1 ml Intramuscular [illegible]ebo	C[illegible]P, IL-[illegible]	CRP ↔ IL-6 ↓	Reduced IL-6 levels

Legend: Female (F); Male (M); interleukin (IL); C-reactive protein (CRP); tumor necrosis factor (TNF); interferon gamma (IFN-@

Table 3: Study quality and risk of bias assessment based on Cochran risk of bias tool(41)

First author, year (ref)	Sequence generation	Allocation concealment	Blinding	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall quality
Amerin. 2014(6)	+	+	+	+	+	+	Good

Leaf. 2014(20)	+	+	+	-	?	+	Good
Nair.2015(22)	+	+	-	-	?	+	Good
Quraishi.2015(23)	+	+	-	-	+	?	Good
Miroliaee.2017(21)	+	+	?	-	?	+	Good

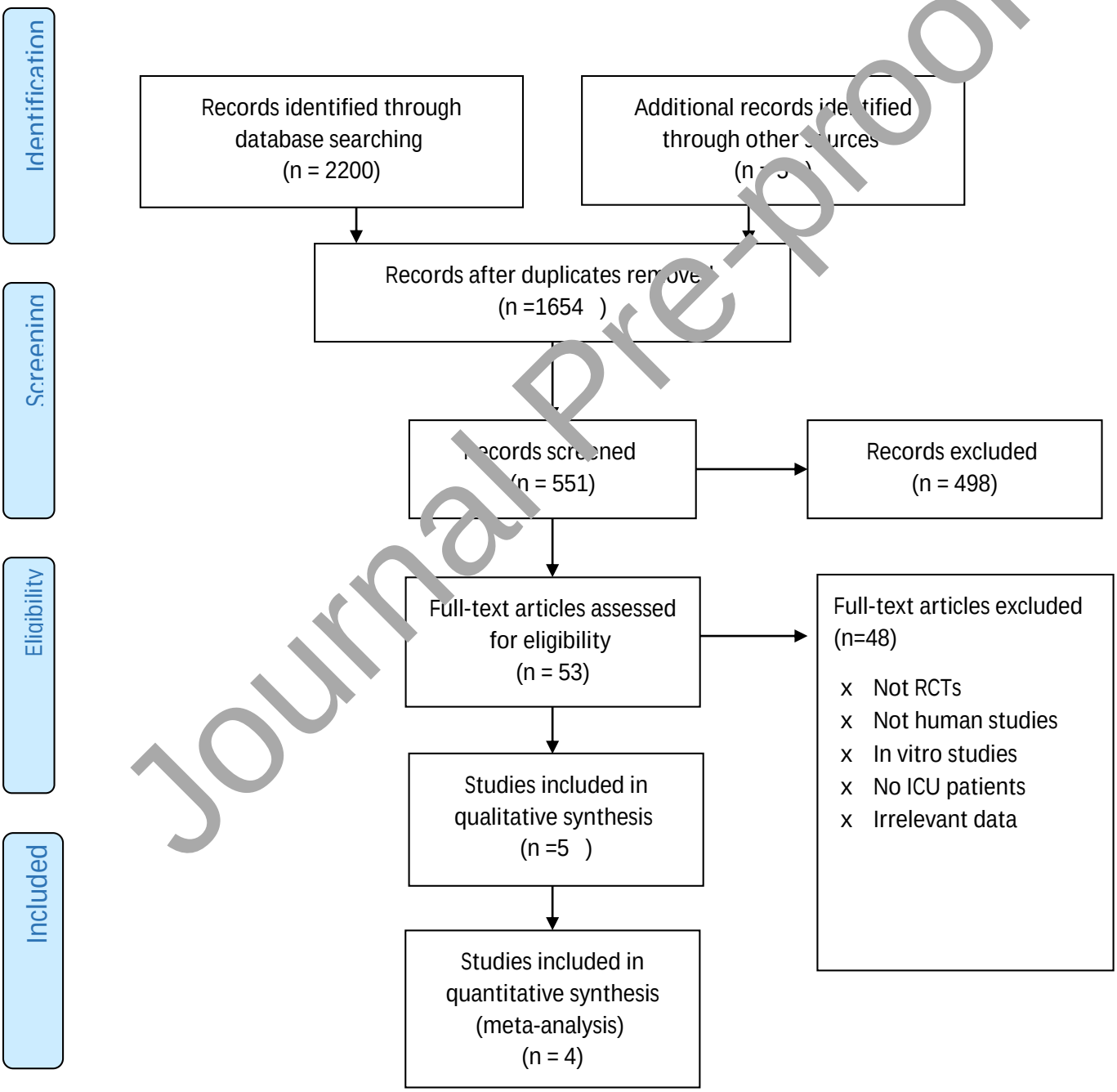

Figure 1: PRISMA flow-diagram of the study selection process.

Figure 2: The effect of vitamin D supplementation on CRP levels. Data is reported as in means difference and 95% CI. (Amerin 1: Mean±SD on day 3, Amerin 2: Mean±SD on day 7)

Figure 3: The effect of vitamin D supplementation on IL-6. Data is reported as in means difference and 95% CI. (Leaf 1: Mean±SD at6 hr., Leaf 2: Mean±SD on day 1, Leaf 3: Mean±SD on day 2)