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EDITORIALE

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VITAMIN D
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Cari Lettori

in questo numero torniamo sul tema del possibile rapporto tra vitamina D e rischio di infezione o di grave manifestazione clinica dell'infezione da SARS-CoV-2.
Lo facciamo perché le pubblicazioni scientifiche su questo topic sono ancora numerose (più di 80 nell'ultimo quadrimestre come potete vedere nella selezione bibliografica) e con risultati talora contradditori, lungi dal consentire conclusioni e tantomeno certezze in un senso o nell'altro, come invece qualcuno si permette di fare, secondo me in maniera inappropriata e imprudente.
Lo facciamo perché il COVID-19 continua tuttora a mietere un numero rilevante di vittime e tante sono ancora le incognite sulla rilevanza sanitaria e sociale delle manifestazioni cliniche delle infezioni da varianti del SARS-CoV-2 nella prossima stagione invernale.
Abbiamo sentito pertanto il bisogno di un aggiornamento e di una sintesi, rigorosa e obiettiva, sullo stato attuale delle conoscenze scientifiche sul tema, compito affidato, com'è consuetudine di questa Rivista, a esperti che ci hanno lavorato e pubblicato.
Nel primo articolo troverete riassunti razionale, evidenze e dubbi sul possibile ruolo della vitamina D nel condizionare il rischio di infezione da SARS-CoV-2 e la severità del COVID-19. L'Autore giustamente parte riassumendo le attuali evidenze sul ruolo fisiologico della vitamina D in ambito di immunità innata, in particolare ad azione antimicrobica, e di immunità acquisita, ad azione modulatrice prevalentemente antinfiammatoria e favorente la tolleranza immunitaria. Poi sintetizza le evidenze sull'associazione tra stato vitaminico D e il rischio specifico di infezione da SARS-CoV-2, incluse quelle indirette come l'alta prevalenza di ipocalcemia nei pazienti ospedalizzati per COVID-19, possibile espressione di disregolazione dell'omeostasi fosfocalcica da carenza di vitamina D, o la ridotta esposizione a raggi UVB, che notoriamente condiziona primariamente lo stato vitaminico D. Da notare che l'esperienza ben pubblicata dallo stesso Autore dell'articolo non ha invece evidenziato alcuna relazione diretta tra indici di esposizione solare (tra cui il confinamento domestico durante il lockdown), livelli sierici di 25(OH)D e infezione da SARS-Cov-2, ammettendo tuttavia la possibile esistenza di altre variabili non considerate. L'Autore giustamente evidenzia la forte dipendenza da co-variabili dell'associazione descritta in numerosi studi tra vitamina D e rischio di infezione (ad es. età avanzata, comorbilità, adiposità, genere, etnia, eventuale supplementazione, peraltro quest'ultima spesso non riportata) per cui la carenza di vitamina potrebbe essere non la causa ma la conseguenza o semplicemente un marcatore di rischio.
Lo stesso dubbio interpretativo caratterizza anche le numerose osservazioni attualmente riportate sulla correlazione inversa tra stato vitaminico D e severità del COVID-19. Tuttavia allo stato attuale delle conoscenze non si può escludere una possibile co-responsabilità della carenza di vitamina D nel condizionare la gravità di alcune manifestazioni cliniche della malattia e i suoi esiti (ospedalizzazione, ricorso alla ventilazione meccanica, trasferimento in terapia intensiva e mortalità). Alcuni possibili meccanismi fisiopatologici attraverso i quali la carenza di vitamina D contribuirebbe alla patogenesi del COVID-19 sarebbero peraltro noti: la vitamina D ha

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la capacità di mitigare la tempesta citochinica e agisce da modulatore endocrino del sistema renina-angiotensina-aldosterone, entrambi coinvolti nella patogenesi dell'*acute respiratory distress syndrome*. Una risposta alle attuali incertezze potrebbe derivare da trial randomizzati e controllati (RCT) di supplementazione con vitamina D, purché ben disegnati da punto di vista razionale e ricordando che la vitamina D potrebbe agire anche in questo campo fondamentalmente come un nutriente e quindi potrebbe rivelarsi utile solo in chi è carente. Le metanalisi dei pochi studi di questo tipo attualmente disponibili sembrerebbero indicare un'efficacia su alcuni outcome, anche se limitata.

È tuttavia innegabile che lo stato attuale delle conoscenze sul tema è caratterizzato da un'ampia variabilità e frequente discrepanza di risultati, tale da meritare una revisione critica della letteratura, che troverete nel secondo articolo di questo numero. Gli Autori vi riassumono alcuni dei maggiori punti deboli delle attuali pubblicazioni disponibili, evidenziando in particolare che gran parte dell'attuale confusione deriva dalla frenesia a pubblicare e dall'utilizzo di strumenti di ricerca inadeguati o di trial mal progettati. Innanzitutto, come riconosciuto anche dall'Autore del primo articolo, il bias della relazione temporale tra dosaggio di vitamina D e diagnosi di COVID-19, che varia nei diversi studi da 1 anno prima alla valutazione contestuale. Ciò appare rilevante anche in considerazione della nota *reverse causality*, cioè del fatto che la malattia stessa, attraverso la condizione di flogosi, si associa di per sé a una riduzione dei valori plasmatici di 25(OH)D. Noterete che la significatività statistica delle correlazioni tra livelli sierici di 25(OH)D e i diversi outcome dipende se il dosaggio è stato fatto prima o in corso di ospedalizzazione. La qualità dei lavori attualmente disponibili è inoltre eterogenea, con diversi studi classificati come *low quality*, essendo esposti a fattori confondenti o mancando di dettagli o di adeguatezza metodologica. Vi è poi il problema del *publication bias*, derivante dalla tendenza a privilegiare la pubblicazione di studi con risultati positivi. Altri problemi che hanno caratterizzato talora la letteratura sul tema del COVID-19 sono la troppa fretta nell'avvallare alcuni dati preliminari, l'eccessiva semplificazione con conclusioni generiche non sostenute dalla significatività statistica e la deroga, considerata la situazione emergenziale, a includere nelle metanalisi di RCT anche studi osservazionali. Ciò ha determinato la pubblicazione di molteplici analisi di bassa qualità, con numerosi fattori confondenti e con risultati di conseguenza contradditori, che hanno esposto la comunità scientifica al rischio di perdita di credibilità. Tutto ciò ha contribuito al persistere tuttora dell'incertezza sull'utilità della supplementazione con vitamina D per la prevenzione e il trattamento del COVID-19. Personalmente temo che un giorno qualcuno concluderà che, anche in questo campo, per la correzione di una carenza, considerati il razionale e la sicurezza, sarebbe bastato utilizzare il buon senso…

Cosa ne pensate ?

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Correlazione tra carenza di vitamina D e COVID-19: revisione critica della letteratura

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Dopo poco più di due anni dopo la dichiarazione da parte dell'Organizzazione Mondiale della Sanità (OMS) dell'*outbreak* di SARS-CoV-2 come pandemia [1], adottando la parola chiave "COVID-19", troviamo su PubMed un numero sorprendente di pubblicazioni (256.087 pubblicazioni, aggiornato al 19 giugno 2022). Seppur in misura minore, anche la ricerca "COVID-19 and vitamin D" restituisce un numero significativo di voci: 1.189. Ciò equivale a circa 1,5 lavori pubblicati quotidianamente; per confronto, "osteoporosis and vitamin D" restituisce 10.914 voci, con tuttavia date delle prime pubblicazioni che risalgono sino agli anni '50.

Effettivamente, l'interesse sulla vitamina D in questo ambito è stato intenso, sin da subito. Già verso fine 2020 pubblicavamo su questa rivista un sunto delle poche evidenze allora disponibili, e in particolare sui primi dati di associazione tra status vitaminico D e rischio di infezione da SARS-CoV-2 [2].

Da allora, moltissimo è stato pubblicato.

In questo articolo riassumeremo le osservazioni tratte da una metanalisi prodotta da esperti Italiani sull'associazione tra status vitaminico D e outcome clinici in pazienti affetti da COVID-19 [3]. Tale metanalisi è disponibile open access, e, se siete interessati all'argomento, vi suggeriremmo di leggerla per esteso. A seguire un breve commento sulla qualità delle evidenze attualmente disponibili sul beneficio della supplementazione in questi pazienti.

STATUS VITAMINICO D E OUTCOME CLINICI: MATERIALI E METODI

Come endopoint primario, l'analisi si è prefissata di sintetizzare l'evidenza disponibile sulla relazione tra status vitaminico D come predittore di gravità di malattia, ovvero mortalità o necessità di trasferimento in terapia intensiva (UTI).

Come endpoint secondario, invece, è stata analizzata la relazione tra status vitaminico D, suscettibilità all'infezione da SARS-CoV-2 e rischio di ospedalizzazione.

Una nota importante: dal momento che la malattia stessa, verosimilmente, si associa a una riduzione dei valori plasmatici di 25-idrossi-vitamina D [25(OH)D] [3], per fare fronte a un bias chiamato *reverse causality* (Fig. 1), sono stati analizzati separatamente gli studi il cui valore di 25(OH)D era stato ottenuto prima dell'infezione (quindi, a rigor di logica, meno condizionati da questo problema), da quelli in cui il valore è stato ottenuto al momento dell'ospedalizzazione.

Di 3.205 voci inizialmente individuate, la selezione ha permesso di includere nell'analisi 54 studi.

Come atteso, in una metanalisi che includa studi osservazionali, la qualità dei lavori selezionati, misurata con la scala di Newcastle-Ottawa (uno strumento apposito per la valutazione degli studi non randomizzati), è risultata piuttosto eterogenea, con diversi studi classificati come *low quality* (punteggio della scala ≤ 6). Gli studi non-randomizzati, come noto, sono infatti esposti all'influenza diversi fattori confondenti. Inoltre, non sempre gli Autori dettagliano e/o includono adeguatamente i metodi con cui sono stati condotti. Tuttavia, l'analisi ristretta a solamente gli studi di qualità elevata (analisi di sensibilità), non ha evidenziato particolari criticità.

Un altro aspetto fondamentale in una metanalisi è la valutazione del *publication bias*, ovvero un fenomeno che si può verificare come conseguenza della realtà editoriale scientifica odierna, che talora privilegerebbe gli studi con risultati "positivi!" (ovvero statisticamente significativi) [4]. L'identificazione di un rischio significativo di *publication bias*, suggerito da test appositi come il test di Egger e l'ispezione del *funnel plot*, è fondamentale per contestualizzare e interpretare i risultati estratti dell'analisi. La Figura 2 mostra due esempi fittizi di *funnel plot*.

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Conflitto di interessi
Gli Autori dichiarano nessun conflitto di interessi.

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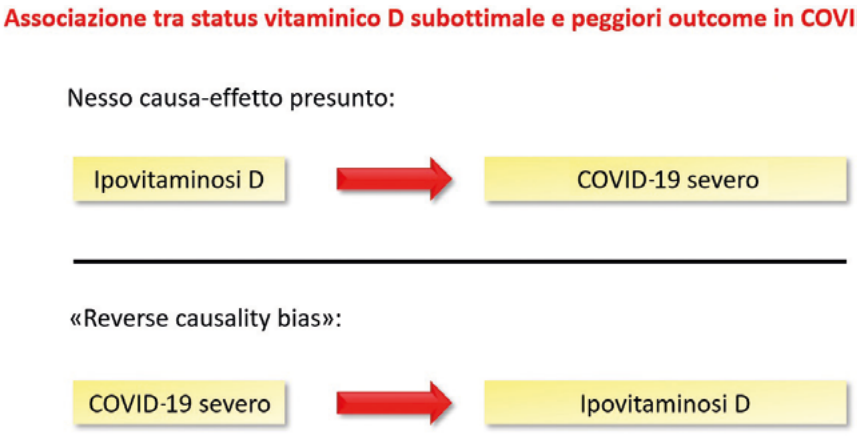

FIGURA 1.
Reverse causality (talora anche denominata *reverse causation*). Bias per il quale la variabile dipendente e quella indipendente vengono scambiate erroneamente.

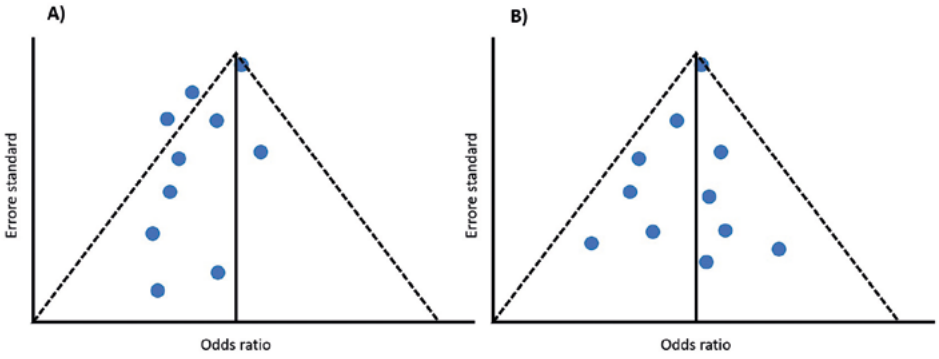

FIGURA 2.
Esempio di *funnel plot* nella valutazione del *publication bias*. Panel A: distribuzione degli studi (ciascuno rappresentato da un pallino blu) chiaramente asimmetrica; *publication bias* probabile. Panel B: non evidente asimmetria; *plot* non suggestivo per *publication bias*.

Nella presente metanalisi, un certo grado *publication bias* è emerso per l'outcome "trasferimento in UTI" quando si è adottata come soglia di 25(OH)D plasmatico < 75 nmol/L e per la mortalità, quando è stata adottata la soglia < 50 nmol/L.
Sulla base di queste osservazioni, non si può pertanto escludere che i risultati ottenuti su questi outcome siano, almeno parzialmente, sovrastimati (sebbene ulteriori analisi eseguite successivamente non abbiano corroborato questo sospetto).

STATUS VITAMINICO D E OUTCOME CLINICI: RISULTATI

Endpoint primario: trasferimento in UTI e mortalità

Il dato probabilmente più eclatante è l'osservazione di un rischio aumentato di necessità di trasferimento in UTI nei pazienti con valori di 25(OH)D < 25 nmol/L, sia per quanto riguarda il dataset di tutti gli 11 studi analizzati [OR (odds ratio) 2,63, 95% IC (intervallo di confidenza) 1,45-4,77], che per quanto riguarda gli studi in cui il dosaggio del 25(OH)D era stato eseguito, per altri motivi, prima dell'ospedalizzazione (OR 2,55, 95% IC 1,28-5,08). Come già menzionato, ciò tenderebbe a escludere la *reverse causality*, dal momento che, in questi studi, il reperto di bassi valori di vitamina D aveva preceduto lo sviluppo della malattia.
Simili risultati, con un incremento del rischio di trasferimento in UTI, sono stati osservati anche per soglie più alte di 25(OH)D (ovvero < 50 e 75 nmol/L), sebbene senza raggiungere la significatività statistica per quanto riguarda l'analisi degli studi in cui 25(OH)D era stata dosata prima dell'ospedalizzazione.
In merito all'outcome "mortalità", il rischio aumentato è stato confermato per tutte le soglie di 25(OH)D (ad es. 25(OH)D < 25 nmol/L, 21 studi, OR per mortalità 2,60, 95% IC 1,93-3,49), ma non nei lavori in cui la vitamina D era stata dosata prima dell'ospedalizzazione.

Endpoint secondari: rischio di infezione da SARS-CoV-2 e ospedalizzazione

Un aumentato rischio di infezione da SARS-CoV-2 è stato confermato per valori di 25(OH)D inferiori a tutte le soglie considerate e confermato per valori di pre-ospedalizzazione < 25 nmol/L e < 50 nmol/L (4 studi, OR 1,42, 95% IC 1,09-1,84 e 3 studi, OR 1,35, 95% IC 1,08-1,69, rispettivamente).
Anche al di sotto dei 75 nmol/L, è stato individuato un rischio di ospedalizzazione aumentato, ma non quando l'analisi è stata ristretta ai valori pre-ospedalizzazione.
Infine, anche per il rischio di ospedalizzazione stessa, l'OR è risultato aumentato per tutte le soglie; tuttavia, solamente per la soglia < 25 nmol/L l'analisi degli studi in cui il dosaggio era stato eseguito prima dell'ospedalizzazione ha riportato un incremento significativo (2 studi, OR 1,99, 95% IC 1,02-3,89).

STATUS VITAMINICO D E OUTCOME CLINICI: COSA POSSIAMO CONCLUDERE

Ancora una volta, anche nel COVID-19 si osserva una forte associazione tra insoddisfacente status vitaminico D, rischio di sviluppare la malattia e peggiori outcome clinici. Come già commentato, per quanto si sia provato a correggere per bias come la *reverse causality*, non è nella natura degli studi osservazionali (da cui questa metanalisi è gemmata), la possibilità di dimostrare un nesso causa-effetto. Gli studi osservazionali possono evidenziare l'associazione tra due variabili, ma essa non implica necessariamente la presenza di un nesso-causa effetto. Come noto, questa capacità è infatti propria degli studi randomizzati e controllati (RCT), di cui discuteremo brevemente in seguito.
Tuttavia, la rigorosa metodologia con cui questa analisi è stata condotta permette comunque di contingentare l'influenza di questi bias e ci restituisce un quadro rias-

suntivo delle evidenze osservazionali a oggi disponibili. Questi risultati suggeriscono pertanto come il deficit di vitamina D rappresenti quantomeno un marcatore di rischio per infezione da SARS-CoV-2 e successiva evoluzione sfavorevole.
In ogni caso, come abbiamo già ampiamente sostenuto in diversi editoriali pubblicati in era pre-COVID-19 [5], non vi sono molti dubbi che il deficit di vitamina D sia da correggere, come da buona pratica medica (ed etica), in particolare per popolazioni a rischio.

SUPPLEMENTAZIONE DI VITAMINA D E STUDI INTERVENTISTICI: METANALISI E CATTIVA SCIENZA

Come già ribadito, solamente la replicazione di dati provenienti da rigorosi RCT può confermare l'utilità di un determinato provvedimento interventistico. All'apice della gerarchia della medicina basata sull'evidenza, infatti, troviamo i risultati di metanalisi basate (esclusivamente) su RCT.
Ciononostante, l'*Handbook* della *Cochrane Collaboration* considera la possibilità di includere non-RCT in una metanalisi, in casi particolari [6]. Una situazione emergenziale come i primi mesi di una pandemia, con la pressante necessità di trovare trattamenti sicuri e potenzialmente efficaci, sicuramente può rappresentare una di queste eccezioni. Tuttavia, dobbiamo ricordare che, per quanto una pandemia possa abbassare l'asticella che rende questo *trade-off* accettabile, l'incertezza e l'influenza dei fattori confondenti che possono affliggere un'analisi di questo tipo rimangono considerevoli e invariate.
Questa pandemia ci ha colto in contropiede, sia come clinici che come ricercatori, e sono stati commessi errori piuttosto clamorosi dovuti a troppa fretta nell'avvallare alcuni dati preliminari; si veda ad esempio il caso del trattamento con idrossiclorochina e azitromicina. Nella prima metà del 2020, dati osservazionali assolutamente preliminari hanno finito comunque per influenzare la pratica clinica di molti di noi, per poi venire completamente smentiti in seguito dai risultati degli RCT [7]. Anche in questo caso, sono emersi i limiti degli studi osservazionali, in cui è spesso impossibile correggere adeguatamente per fattori confondenti (contestuali e umani) con il rischio significativo di restituire dati di efficacia alterati e sovrastimati [7].
Credo sia l'auspicio di tutti noi quello di evitare il replicarsi di una situazione analoga per quanto riguarda la supplementazione di vitamina D nel COVID-19.
A oggi, sono presenti solamente 6 RCT sulla supplementazione con vitamina D e outcome clinici [8–13] (Tab. I). Peraltro, per la maggior parte di questi, l'outcome clinico non rappresentava l'endpoint primario e non sono stati pertanto progettati per esso. Di questi 6 RCT, solamente 2 (affetti comunque da significativi limiti metodologici) [8,9] parrebbero individuare un qualche segnale di efficacia. Un settimo studio è stato addirittura retratto dopo la pubblicazione per le gravi criticità da cui era afflitto: Lakireddy et al. [14].
D'altro canto, tramite una veloce ricerca su PubMed, possiamo ritrovare almeno 10 review sistematiche con metanalisi (che non elencheremo, per non appesantire eccessivamente il documento). Ovviamente, la maggior parte di queste metanalisi ha incluso anche, se non solamente, studi osservazionali. Credo sia importante rimarcare come questo modo di procedere, e questo proliferare di analisi riassuntive di dati di qualità inadeguata, metta la comunità scientifica a rischio di una grave perdita di credibilità, in particolare oggi che abbiamo trattamenti supportati da RCT e dalle raccomandazioni internazionali [15].
Ad esempio, una di queste metanalisi [16] (definibile *umbrella meta-analysis*, in quanto a sua volta riassuntiva di altre 7 review sistematiche con metanalisi – tutte di studi osservazionali) suggerirebbe una riduzione di addirittura il 50% della mortalità da COVID-19 nei pazienti trattati con vitamina D (OR 0,479, 95% IC 0,346-0,664). Per meglio contestualizzare il tutto, nessuno dei trattamenti considerati dalle raccomandazioni dell'*European Society of Clinical Microbio-*

TABELLA I.
Tabella riassuntiva degli studi randomizzati e controllati a oggi disponibili sul trattamento di COVID-19 con vitamina D.

Riferimento	Paese	Numerosità campionaria	Intervento e durata	Risultati
Sabico, Nutrients 2021	Arabia Saudita	69	5.000 *vs* 1.000 UI di D_3 per 2 settimane	Il gruppo trattato ha mostrato un recupero più veloce in termini di risoluzione della tosse e ageusia
Murai, JAMA 2021	Brasile	240	200.000 UI D_3 (dose singola) *vs* placebo	Non differenze significative in termini di mortalità ospedaliera, trasferimento UTI o necessità di ventilazione meccanica
Castillo, J Steroid Biochem Mol Biol 2020	Spagna	76	Calcifediolo 0,532 mg al giorno 1, 0,266 mg al giorno 3 e 7, in seguito settimanale fino alla dimissione dall'UTI *vs* placebo	Il gruppo trattato ha avuto una riduzione significativa del rischio di trasferimento in UTI
Maghbooli, Endocr Pract 2021	Iran	106	Calcifediolo 25 µg/die per 60 giorni *vs* placebo	Nessuna differenza significativa negli outcome clinici
Elamir, Bone 2022	Iran	50	Calcitriolo 0,5 µg/die per 2 settimane *vs* placebo	Nessuna differenza significativa negli outcome clinici; riduzione statisticamente significativa dell'utilizzo di ossigeno nel gruppo trattato
Cannata Andia, BMC Med 2022	Spagna	543	Bolo singolo di 100.000 UI di D_3 *vs* placebo	Nessuna differenza significativa negli outcome clinici

D_3: colecalciferolo; UTI: unità di terapia intensiva; UI: unità internazionali.

logy and Infectious Diseases [15] presenta un *effect size* che si avvicini anche solo lontanamente a un tale dato. Capite come un risultato simile sia davvero poco credibile e come rappresenti il tipico esempio del detto: *garbage in, garbage out* ("spazzatura dentro, spazzatura fuori"). Non importa quanto potente e raffinato possa essere il nostro strumento (in questo caso, la metodica metanalitica): se la qualità dei dati è pessima, lo sarà pure l'output finale.
Ahimè, a oggi, nemmeno chi si è prefissato di eseguire analisi di natura più selettiva ha dimostrato un sufficiente rigore metodologico. Ad esempio, Rawat et al. [17], come specificato nella sezione materiali e metodi della loro metanalisi, avrebbero incluso solamente RCT e studi "quasi-sperimentali". Tuttavia, classificare come "quasi-sperimentali" tali studi pare quantomeno controverso: tali lavori presentavano semplicemente un design osservazionale, e come tali sono infatti regstrati su clinicaltrials.gov.
Credo infine che valga la pena di segnalare la review sistematica con metanalisi di Varikasuvu et al. [18], pubblicata su *Expert Review of Anti-infective Therapy* (un giornale con più di 5 di *impact factor*). Gli Autori di questa review che, in effetti, ha incluso esclusivamente RCT, concludono che i pazienti COVID-19 supplementati con vitamina D hanno una maggiore probabilità di esitare in minori tassi di trasferimento in terapia intensiva, mortalità e positività al tampone RT-PCR.
Eppure, esaminando il paper, emergono degli strani aspetti.
Innanzitutto: come interpretereste queste conclusioni? Immagino, assumereste come significativo il beneficio in termini di trasferimento in UTI, mortalità e per positività al tampone. Eppure, ad esempio, l'analisi della mortalità non fornisce assolutamente un risultato significativo: OR 0,78, 95% IC 0,25-2,40.
Innanzitutto, crediamo valga la pena di segnalare come diversi studi siano stati inclusi svariate volte all'interno della stessa analisi (ad es. nell'analisi della "severità" uno stesso studio è stato incluso sia per il parametro "ventilazione meccanica" che "trasferimento in UTI"). Oltre a ciò, va segnalato come il risultato "significativo" citato nella (a nostro modo di vedere ambigua) conclusione degli autori è, in realtà, riferito all'analisi complessiva di tutti gli outcome accorpati.

Ovvero: la significatività ottenuta (un OR 0,6, 95% IC 0,4-0,92) è stata ottenuta raggruppando tutti assieme i dati relativi a: "COVID-19 severity" (severità), "RT-PCR positivity" (positività della real-time PCR), "COVID-19 seropositivity" (sieropositività) e "Deaths" (mortalità).

Sebbene nelle conclusioni del full-text questo aspetto sia stato menzionato, riteniamo che, per correttezza, avrebbe dovuto esserlo anche nell'abstract, ovvero il primo elemento che viene considerato dai fruitori.
Infine, questo studio ha purtroppo incluso anche lo studio di Lakireddy, già menzionato, in quanto soggetto di *retraction* dopo la pubblicazione. Nell'analisi del *risk of bias* della suddetta metanalisi (consultabile come materiale supplementare), era stato valutato come affetto da *some concerns* ("qualche criticità"), giudizio a ogni modo sufficiente per l'inclusione nell'analisi. Nello specifico, peraltro, sotto la voce *randomisation process* ("processo di randomizzazione"), il giudizio degli autori era stato completamente positivo (luce verde).

CONCLUSIONI

L'ipovitaminosi D è una condizione problematica e diffusa. A fronte di una mole impressionante di studi epidemiologici che ne hanno evidenziato l'associazione con moltissime condizioni patologiche [5], l'utilizzo di strumenti di ricerca inadeguati o di trial mal progettati [5] ha creato molta confusione tra i clinici sulle modalità e le indicazioni per la sua supplementazione.
Qualcosa di analogo sta accadendo anche nell'ambito COVID-19, in cui l'associazione tra malattia grave e ipovitaminosi D è stata confermata da dati ormai solidi, mentre la presenza di un reale beneficio della supplementazione una volta che la malattia si è sviluppata rimane ancora un'incognita. Personalmente, ritengo che sia compito di tutti noi vigilare affinché la qualità della ricerca in questo ambito rimanga adeguata, per evitare che ulteriori studi affetti da pesanti limitazioni metodologiche non contribuiscano a creare ulteriore confusione.

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Vitamina D, rischio di infezione da SARS-CoV-2 e severità COVID-19: dubbi, possibilità ed evidenze

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INTRODUZIONE

Vitamina D è un regolatore chiave di sviluppo e maturazione di tutti i *lineages* immunitari. La supplementazione, in caso di carenza, ha mostrato effetti positivi verso le infezioni respiratorie acute, sebbene non riduca l'incidenza di eventi seri.

Molti report, basati sulle osservazioni effettuate durante la prima ondata pandemica in Italia, hanno suggerito l'associazione tra carenza di vitamina D, rischio di infezione da SARS-CoV-2, incidenza e severità di COVID-19, mortalità. Osservazioni speculative, che mettevano in relazione la più alta prevalenza di ipovitaminosi D tra i paesi europei e l'altissima prevalenza di infezioni SARS-CoV-2 e COVID-19 in Italia, e soprattutto nelle regioni settentrionali, hanno definito l'associazione tra i due eventi senza verificarne il nesso di causalità e senza escluderne la casualità. Status vitaminico D, rischio di infezione e sviluppo di forme gravi della patologia sono fenomeni complessi dipendenti da innumerevoli variabili la cui complessa relazione di interdipendenza non può essere descritta dalla loro mera sommatoria. Pertanto, solo studi svolti in ampie coorti e non prescindenti da variabili fondamentali possono assumere una rilevanza epidemiologica [1].

RUOLO DELLA VITAMINA D NELLE RISPOSTE IMMUNITARIE INNATA E ADATTATIVA

La vitamina D ha ruoli rilevanti in ambito di immunità innata, attraverso l'azione antimicrobica [regolazione di metabolismo marziale, autofagia e funzione barriera degli epiteli, stress ossidativo, espressione di composti ad attività antimicrobica (defensine, catelicidine) e *toll-like receptors*], di modulazione della risposta adattativa e induzione di tolleranza [1].

Più nello specifico, l'$1,25(OH)_2D$ svolge, di per sé, attività antimicrobica in quanto in grado di indurre l'espressione di catelicidina e β-defensina 2, proteine con efficacia antimicrobica sia diretta che indiretta (attraverso la stimolazione della chemiotassi delle cellule del sistema immunitario, inducendo l'espressione di citochine pro-infiammatorie e determinando la rimozione di cellule infette nel tratto respiratorio). L'espressione di β-defensina 2 è stimolata dalla vitamina D anche attraverso l'induzione di nucleotide-*binding oligomerization domain-containing protein* (NOD2) [2]. Inoltre, l'$1,25(OH)_2D$ inibisce l'espressione di epcidina e determina, pertanto, l'eliminazione del blocco epcidina-mediato dell'esportazione di ferro dipendente da ferroportina: il risultato netto è l'aumentato efflusso di ferro dalla cellula infettata e, conseguentemente, la riduzione della disponibilità di questo elemento per la crescita microbica [3]. A onore del vero, gli effetti antimicrobici della vitamina D sono molteplici e includono, anche, la stimolazione della funzione barriera degli epiteli intestinale [4] e alveolare [5], della produzione di specie reattive dell'ossigeno (ROS) [6], della funzione neutrofilica [7] e delle attività fagocitiche e autofagocitiche (attraverso l'induzione degli effettori chiave dell'autofagia: LC3, beclina 1 e PI3Kγ3) dei macrofagi [8]. Sia l'induzione di catelicidina e defensine, sia la stimolazione delle vie pro-autofagiche in cellule presentanti l'antigene hanno un importante effetto anti-virale e, rispettivamente, di inibizione della replicazione dei virus [9] e di aiuto nella *clearance* delle particelle virali [10].

In ambito di immunità adattativa, il calcitriolo limita l'attivazione dei linfociti T [11] e induce l'espressione di fenotipi regolatori (Treg) che mediano la tolleranza immunitaria e limitano risposte immuni abnormi e lo *shift* fenotipico

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da T helper Th1/Th17 a Th2 (da pro-infiammatorio a regolatorio) [12].
L'efficacia dell'azione della vitamina D è funzione dell'attività del suo recettore, VDR. Infatti, polimorfismi a singolo nucleotide (SNPs) nel gene *VDR* hanno effetti sulla responsività della proteina e sono stati associati a numerose disfunzioni immunitarie: rispetto ai genotipi CT e CC, il genotipo TT del polimorfismo FokI, ad esempio, è stato associato a un maggior rischio di infezioni da virus respiratorio sinciziale (SCV) [13].

VITAMINA D E RISCHIO DI INFEZIONE DA SARS-CoV-2

L'ipotesi di un ruolo della vitamina D nella suscettibilità all'infezione da SARS-CoV-2 deriva, in parte, dall'osservazione dell'alta prevalenza di ipocalcemia (50%) tra i pazienti ospedalizzati durante le epidemie di Ebola (2016) e SARS (2003). Fino all'80% dei pazienti COVID-19 ospedalizzati in Italia, durante la prima ondata, riportava $[Ca^{2+}] < 1,18$ mmol/L. Il calcio libero è necessario per interazione virus-cellula (via proteina spike e ACE2), replicazione virale e risposta infiammatoria all'infezione. L'associazione tra status vitaminico D e rischio di infezione potrebbe essere, almeno in parte, conseguente alla deregolazione dell'omeostasi (fosfo)calcica [14]. Che il calcio sia fondamentale nel processo di infezione è dimostrato, tra le altre cose, dal fatto che il blocco farmacologico dei canali del calcio *L-type* rallenta la velocità di replicazione del deltacoronavirus suino [15].
Il calcio libero intracellulare è richiesto durante la risposta a SARS mediata dal complesso dell'inflammasoma NOD-, LRR- e *pyrin domain-containing protein 3* (NLRP3) [16,17]. Durante l'infezione da coronavirus, incluso SARS-CoV-2, il calcio media la fusione dell'*envelope* virale con la membrana della cellula ospite: le subunità S1/S2 della proteina virale di fusione spike (S) interagiscono in maniera calcio-dipendente con il macchinario endocitico della cellula ospite e/o con il dominio transmembrana di ACE2 (*angiotensin-converting enzyme 2*), il designato recettore di SARS-CoV-2 espresso dalle cellule degli epiteli alveolare, intestinale e tubulare renale, da cardiomiociti e cellule endoteliali [18,19].
Numerosi sono gli articoli pubblicati a supporto dell'ipotesi dell'esistenza di una associazione tra status vitaminico D e rischio di infezione da SARS-CoV-2. Tra i primi studi in questo senso, una ricerca statunitense, che ha preso in analisi 191.779 soggetti, nel trimestre compreso tra metà marzo e metà giugno 2020, ha evidenziato una forte associazione anche dopo aggiustamento per fattori demografici (quali latitudine, etnia, genere, età) [20].
Una recentissima revisione sistematica identifica una relazione inversa tra basse temperature, indice UV, *cloud-free vitamin D UV dose* (UVDVF) e prevalenza di COVID-19, in Europa [21]. Di contro, un nostro studio (2021), condotto su 101.035 soggetti dell'area di Milano, che ha posto in relazione il periodo pre-pandemico (2019) e i periodi comprendenti le cosiddette "prima" (gen-ago 2020) e "seconda ondata" (giu-nov 2020), non ha evidenziato alcuna relazione diretta tra indici di esposizione solare, livelli di 25(OH)D e infezione da SARS-CoV-2. Inoltre, non ha identificato alcuna relazione tra 25(OH)D e confinamento domestico durante i lockdown presupponendo all'esistenza di altre variabili non considerabili [22].
Rilevanti sono gli studi effettuati sulle casistiche da biobanca: in 348.598 partecipanti alla *UK Biobank* (37-73 anni), l'associazione tra 25(OH)D e rischio di infezione viene persa dopo aggiustamento per fattori confondenti e provenienza etnica [23,24]. Fattore rilevante, ma spesso non riportato negli studi, è la supplementazione.
Data la grande quantità (e varietà) di studi sull'argomento, è necessario il supporto delle metanalisi. Buona parte di esse identifica un'associazione inversa tra deficienza di vitamina D e rischio di infezione da SARS-CoV-2 (Tab. I). Alcuni di questi report, però, evidenziano la forte dipendenza di questa associazione da altre variabili come l'età avanzata, comorbidità (es. diabete, ipertensione, adiposità) e, eventualmente, l'appartenenza al genere maschile. Emerge, pertanto, l'impossibilità di stabilire se la carenza vitaminica rappresenti una causa dell'aumentato rischio di infezione o, piuttosto, rifletta (o sia conseguente a) una condizione fisiopatologica che di per sé aumenta il rischio di infezione.

VITAMINA D E SEVERITÀ COVID-19

Lo scenario attuale suggerisce l'associazione tra ipovitaminosi D e severità di COVID-19 ma è altresì evidente che comorbidità ed età hanno un ruolo decisamente più rilevante. Ciononostante, l'ipovitaminosi D cronica può predisporre allo sviluppo di comorbidità ed essere, pertanto, determinante più o meno indiretto della severità della malattia: difatti, età avanzata e sovrappeso sono associati sia a decorso COVID-19 più severo sia a ipovitaminosi D [36].
Per la vitamina D è stato ipotizzato un ruolo in *acute respiratory distress syndromes* (ARDS). ACE2, che come riportato in precedenza funge da sito di attracco per la proteina virale S, è un enzima che converte angiotensina II (Ang-II) in angiotensina 1-7 [Ang(1-7)]. Quest'ultima ha azione vasodilatatrice, antinfiammatoria e di protezione dal danno d'organo [37]. A seguito del legame con la proteina S, il complesso ACE2-particella virale viene internalizzato e, pertanto, l'attività enzimatica di ACE2 risulta down-regolata. La down-regolazione di ACE2 è associata a una risposta infiammatoria abnorme che può causare danno tissutale che determina, a sua volta, ulteriore down-regolazione di ACE2. Questo processo può esitare nella sindrome acuta da distress respiratorio (ARDS) [38,39]. La vitamina D ha un ruolo protettivo verso ARDS data la capacità di inibire l'espressione di renina e dell'attività dell'asse ACE/Ang-II/AT1R e di stimolare, invece, l'asse ACE2/Ang-(1-7)/MasG (recettore Mas associato a proteina G). Pertanto, la vitamina D agisce da modulatore endocrino negativo del sistema renina-angiotensina-aldosterone (RAAS) [40,41]. Proprio la risposta infiammatoria abnorme (tempesta citochinica), conseguente all'infezione da SARS-CoV-2, è responsabile dello sviluppo di COVID-19 e, in alcuni casi, di manifestazioni di severità crescente [42].
La cosiddetta "tempesta citochinica", caratterizzata dal massivo e sostenuto rilascio di citochine pro-infiammatorie (IL-1, IL-6, TNFα, IFNγ), è responsabile dei sintomi e del danno d'organo (a carico, soprattutto, di polmoni e cuore). Di queste citochine, IL-6 è risultata associata a prognosi e mortalità in casi COVID-19 severi (livelli circolanti 2,9 volte quelli registrati nei casi meno gravi). I dati disponibili supportano il ruolo della vitamina D nel mitigare la tempesta citochinica attraverso l'induzione di mediatori antinfiammatori (IL-10, IL-4, TGFβ). Inoltre, come descritto in precedenza, l'induzione da parte di $1,25(OH)_2D$ dell'espressione dei fenotipi a funzione più propriamente antinfiammatoria e regolatoria Th2 e T-reg, a spese di quelli pro-infiammatori Th1/Th17 e più prominentemente coinvolti nello *storm* citochinico, potrebbe avere un effetto nel mitigare la risposta iperinfiammatoria e, pertanto, le manifestazioni di COVID-19 [14].
Uno studio Iraniano, basato su dati raccolti durante la prima ondata (fino a maggio 2020), riporta che dei pazienti ospedalizzati

TABELLA I.
Sommario dei risultati ottenuti da metanalisi relative all'associazione tra livelli circolanti di 25(OH)D e rischio di infezione da SARS-CoV-2.

N. studi inclusi Disegno degli studi	Pazienti	Associazione con rischio di infezione	Data analisi	Ref.
8 CS, Re-Co	≤ 18 anni Europa, Nord America	Sì (deficienza)	06-2021	25
13 Co, RCT	49-69 anni (età media) Asia, Australia, Europa, Nord America, Sud America	No (insufficienza, deficienza)	06-2021	26
72 CC, CS, Os, Pr-Co, Re-Co, RCT	/ Asia, Europa, nord Africa, Nord America, sud America	Sì	05-2021	27
49 Pr, Re	35-85 anni (età media/mediana) Asia, Europa, Nord America, Sud America, Nord Africa	Sì (deficienza grave, deficienza, insufficienza)	03-2021	28
43 CC, CS, Os, Pop, Pr, Pr-Co, Re, Re-CC, Reg	35-90 anni (mediana età) Asia, Europa, Nord Africa, Nord America	Sì (deficienza)	01-2021	29
21 CC, CS-Co	47-81 anni (età media) Asia, Europa, Nord America	Sì	12-2020	30
23 Re	35-77 anni (età media/mediana) Asia, Europa, Nord America	Sì (deficienza)	12-2020	31
14 CS, Pr-Os, Re-Os	46-81 anni (età media/mediana) /	Sì	12-2020	32
34 CC, Co, CS, RCT	42-88 anni (età media) Europa, Asia, Nord America	No	12-2020	33
14 CC, Co, CS	/ Asia, Europa, Nord America	Sì (insufficienza)	12-2020	34
10 CC	Asia, Europa, Nord America	Sì (deficienza, insufficienza)	9-2020	35

CC: studio caso-controllo; Co: studio di coorte; CS: studio *cross-sectional*; Os: studio osservazionale; Pop: studio di popolazione; Pr: studio prospettico; RCT: trial randomizzato controllato; Re: studio retrospettivo; Reg: registro di popolazione.

per COVID-19, il 74% presentava una malattia severa e, di questi, il 32,8% presentava uno status vitaminico D adeguato. Livelli sufficienti di vitamina D associavano con una condizione clinica meno severa, riduzione della mortalità, livelli di CRP più bassi e una conta relativa di linfociti più elevata. Dei pazienti deceduti, di età superiore ai 40 anni, solo il 9,7% presentava livelli sufficienti di vitamina D contro il 20% che, invece, presentava livelli < 30 ng/mL [43]. Dello stesso periodo è uno studio Italiano che ha preso in considerazione 61 pazienti ospedalizzati per COVID-19 evidenziando che il 72,1% di essi presentava livelli di 25(OH)D < 20 ng/mL (di cui il 57,4% addirittura inferiori a 15 ng/mL). I valori di pressione parziale di ossigeno arterioso e CRP, come pure la severità della patologia, sono risultati associati allo status vitaminico D [44].

Anche l'ipocalcemia conseguente a ipovitaminosi D è stata associata a una prognosi peggiore. L'ipocalcemia è risultata più frequente in maschi e anziani e i livelli di calcio sono risultati inversamente associati a CRP, LDH e rischio di ricovero in terapia intensiva (ICU). Inoltre [Ca^{2+}] < 2,00 mmol/L all'ammissione associavano con condizioni cliniche peggiori, incidenza di danno d'organo e shock settico e mortalità a 28 giorni. La concentrazione di calcio sierico ha, effettivamente, un valore prognostico definito da una AUC di 0,73 [1].

Come si evince dalla raccolta di metanalisi (Tab. II), se livelli bassi di vitamina D sembrano associare con una sintomatologia più severa e un maggior rischio di ospedalizzazione, meno definita è, invece, l'associazione con altri outcome, e in particolare con rischio di ricorso alla ventilazione meccanica, ammissione a ICU e mortalità. In una delle revisioni sistematiche più recenti, che include 20 studi e 12.806 pazienti di età compresa tra 42 e 81 anni, non è stata riscontrata alcuna differenza tra soggetti ipo- e normovitaminosici in fatto di mortalità, ammissione a ICU, supporto alla ventilazione e durata dell'ospedalizzazione [45]. Similmente, l'analisi di 6 studi e 1.424 pazienti, non evidenzia alcuna differenza nei livelli di 25(OH)D tra pazienti COVID-19 severi e non severi, come pure nessuna associazione con la mortalità [46].

È evidente l'ampia varietà, e la discrepanza, di risultati tra i vari studi e i relativi *bias*. Uno fra tutti è la relazione temporale tra dosaggio di vitamina D e diagnosi di COVID-19 che, nei diversi studi, varia dai 12 mesi antecedenti alla valutazione contestuale.

Riguardo l'utilità della supplementazione con vitamina D, una metanalisi di 6 RCT e 551 pazienti COVID-19 supporta l'efficacia dell'intervento in termini di accesso a ICU, mortalità e positività al test PCR [49]. Risultati simili emergono da una metanalisi di revisioni sistematiche [50]. Dalle metanalisi pubblicate (a giugno 2022) emerge, invece, che l'intervento di supplementazione ha una efficacia limitata (Tab. III). Anche in questo caso, la grande varietà nel disegno

TABELLA II.
Sommario dei risultati ottenuti da metanalisi relative all'associazione tra livelli circolanti di 25(OH)D e outcome clinici di COVID-19.

N. studi inclusi Disegno degli studi	Pazienti	Associazione inversa con outcome					Data analisi	Ref.
		Severità/ ospedalizzazione	Durata malattia/ ospedalizzazione	Ventilazione meccanica	Ammissione ICU	Mortalità		
13 Co, RCT	49-69 anni (media) Asia, Australia, Europa, Nord America	/	/	/	No	No	06-2021	26
8 CS, Re-Co	≤ 18 anni Europa, Nord America	Sì	/	/	/	/	06-2021	25
72 CC, CS, Os, Pr-Co, Re-Co, RCT	Asia, Europa, Nord Africa, Nord America, Sud America	Sì	/	/	/	Sì	05-2021	27
49 Pr, Re	35-85 anni (media/mediana) Asia, Europa, Nord America, Sud America, Nord Africa	Sì	/	/	Sì	Sì	03-2021	28
8 Po	/ Asia, Europa, Nord America	/	/	/	/	Sì	03-2021	47
43 Co, CS, Os, Os-CC, Pr, Re, Re-CC, Reg	35-90 anni (mediana) Asia, Europa, Nord Africa, Nord America	Sì	/	/	/	Sì	01-2021	29
21 CC, CS	47-81 anni (media) Asia, Europa, Nord America	Sì	/	/	/	No	12-2020	30
23 Re	35-77 anni (media/mediana) Asia, Europa, Nord America	Sì	/	/	/	No	12-2020	31
17 Os	/ Europa, Asia, Medio Oriente, Nord America	Sì	Sì	/	No	Sì	12-2020	48
14 CS, Pr-Os, Re-Os	46-81 anni (media/mediana) /	Sì	/	/	/	Sì (♂, diabete, ipertensione)	12-2020	32
34 CC, Co, CS, RCT	42-88 anni (media) Europa, Asia, Nord America	No	No	No	No	No	12-2020	33

CC: studio caso-controllo; Co: studio di coorte; CS: studio *cross-sectional*; Os: studio osservazionale; Pop: studio di popolazione; Pr: studio prospettico; RCT: trial randomizzato controllato; Re: studio retrospettivo; Reg: registro di popolazione.

degli studi e delle coorti considerate rende difficile trarre delle conclusioni generali.
Dal punto di vista fisiologico, merita attenzione *vitamin D binding protein* (VDBP) che, oltre a legare ad alta affinità l'1,25(OH)$_2$D, interviene nella regolazione della risposta immunitaria innata e neutralizza G-actina libera, rilasciata in grandi quantità a seguito di morte cellulare in ARDS e stimola potente risposta infiammatoria, coagulazione intravascolare, degranulazione vescicolare, chemoattrazione leucocitaria [51].

VITAMINA D ED EFFICACIA VACCINALE

L'introduzione di trattamenti efficaci nel prevenire le forme COVID-19 severe, e in particolare i vaccini, è stata il punto di svolta. A oggi mancano report sull'associazione tra livelli di vitamina D (ed eventuale supplementazione) e l'efficacia vaccinale. L'associazione positiva tra 25(OH)D e titolo anticorpale è stata rilevata in uno studio britannico dopo 8 settimane dalla prima dose di BNT162b2 [58] ma non in una popolazione greca dopo seconda dose[59].

CONCLUSIONI

Nonostante le numerose osservazioni, l'associazione causa-effetto tra status vitaminico D, rischio di infezione da SARS-CoV-2 e severità di COVID-19 non è stata stabilita. È ragionevole supporre che uno status vitaminico D adeguato rispecchi una omeostasi bilanciata e, pertanto, favorisca una risposta più efficace all'infezione [14]. A supporto, una recente revisione sistematica identifica la deficienza di micronutrienti, inclusi calcio e vitamina D, come variabile rilevante per rischio di ammissione a ICU, intubazione e morte [60]. Altri Autori sostengono che, data la comprovata sicurezza della supplementazione con vitamina D, la sola possibilità di una associazione giustifica l'implementazione di protocolli di trattamento.
Un aspetto poco considerato, ma meritevole di approfondimento in termini preventivi di eventuali future epidemie, è rappresentato dall'effetto di insufficienza/deficienza croni-

TABELLA III.
Sommario dei risultati ottenuti da metanalisi relative agli effetti della supplementazione con vitamina D su rischio di infezione da SARS-CoV-2 e outcome clinici di COVID-19.

N. studi inclusi Disegno degli studi	Pazienti e intervento	Associazione inversa con outcome						Data analisi	Ref.
		Rischio infezione	Severità/ ospedalizzazione	Durata malattia/ ospedalizzazione	Ventilazione meccanica	Ammissione ICU	Mortalità		
23 Co, Pr-Co, Re, Re- CC, Re-Co, RCT	15-103 anni Asia, Europa, Nord America, Sud America Prevenzione primaria Prevenzione secondaria Prevenzione terziaria	 No / /	 No n.d. /	 / / /	 / / /	 / / Sì	 / / Sì	01-2022	33
8 Os, RCT	53-88 anni (media) Asia, Europa, Sud America	/	/	/	Sì	Sì	No	07-2021	52
2 RCT	49-69 anni (età media) Europa, Sud America	/	/	/	/	No	No	06-2021	26
13 CC, Co, CS, Os, Pr, Re, RCT	45-90 anni (media, mediana) Asia, Europa, Sud America Supplementazione pre-diagnosi e/o post-diagnosi	/	/	/	/	Sì	Sì	06-2021	53
4 CC, CS, EKO, Re-Co	49-74 anni (mediana) Europa	/	No	/	/	/	No	06-2021	54
5 Os, RCT	53-88 anni (media) Asia, Europa, Sud America Supplementazione dopo diagnosi	/	/	/	No	No	No	05-2021	55
10 Co, CS, Os, Pr-CS, Re, Re-CC, Re-Os, RCT	53-88 anni (media) Europa, Asia, Sud America Supplementazione post-diagnosi • Alte dosi • Basse dosi	 / /	 / /	 / /	 / /	 No Sì	 No Sì	03-2021	56
3 Re-CC, RCT	Europa, Sud America	/	/	/	/	Sì	No	12-2020	57

CC: studio caso-controllo; Co: studio di coorte; CS: studio *cross-sectional*; EKO: studio ecologico; Os: studio osservazionale; Pr: studio prospettico; RCT: trial randomizzato controllato; Re: studio retrospettivo.

ca che potrebbe rappresentare una causa, o concausa, più plausibile della carenza attuale, delle disfunzioni alla base dell'aumentato rischio di eventi avversi.

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